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CHANGES IN THE CHEMISTRY OF CEREBROSPINAL FLUID DURING ENCEPHALOGRAPHY*

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THE widespread use of encephalography and ventriculography in the diagnosis and prognosis of pathologic conditions of the central nervous system has stimulated the study of the effects of withdrawal of large amounts of cerebrospinal fluid. It has been found that there is usually a pleocytosis in the cerebrospinal fluid subsequent to encephalography[1, 2] and that the sugar content is usually increased toward the end of the procedure. There has been some disagreement as to whether or not the protein content of the cerebrospinal fluid is affected by the displacement of fluid by air. According to the recent work of Schwab and von Storch,[2] the protein content decreases slightly during the course of the procedure and returns to normal toward the end.

In examining the literature on the subject of the cerebrospinal fluid reaction to encephalography, it becomes apparent that only a very few constituents of the cerebrospinal fluid have been studied to any extent, viz., cells, protein, and sugar. Furthermore, most investigations have been carried out after encephalography, while very little work has been done on the changes which occur in the cerebrospinal fluid during the course of the procedure. It appeared worth while, therefore, to study the changes, if any, of a number of chemical constituents of the cerebrospinal fluid during the course of encephalography. Accordingly, we have studied the effect of displacement of cerebrospinal fluid by air on the sugar, chloride, nonprotein nitrogen, total protein, calcium, and phos-

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TABLE I

ENCEPHALOGRAPHY

CASE	DIAGNOSIS	AMOUNT OF AIR INJECTED (C.C.)	CELL COUNT	PHOSPHATASE (UNITS)	TRYPSIN (UNITS)	ANTITRYPSIN (UNITS)	LIPASE (UNITS)	TRIBUTYRINASE (UNITS)	ESTERASE (UNITS)	AMYLASE	SUGAR	CHLORIDES	TOTAL PROTEIN	CALCIUM	PHOSPHORUS
											MG. PER 100 C.C. OF FLUID				
L. G.	Epilepsy	0	0	1.3	0	0	0	0	0	0.5	58	738	15	5.6	1.9
		25		1.1	0	0	0	0	0	0.5	60	726	14	5.1	1.9
		45		1.2	0	0	0	0	0	0.5	61	721	14	5.1	1.9
		60		2.0	0	0	0	0	0	0.5	63	721	14	5.3	1.9
		80		2.5	0	0	0	0	0	0.5	63	727	18	5.2	1.9
M. R.	Cerebral agenesis with mild hydrocephalus	0	1	1.1	0	7	0.4	1.3	0	0	53	724	17	5.4	2.1
		40	--	2.8	0	0	0.3	1.9	0	0	55	718	19	5.5	2.2
		60	--	3.5	--	--	0.5	0.8	0	--	57	711	25	--	2.2
		80	6	3.8	0	0	0.5	1.0	0	0	63	711	27	5.5	2.2
M. G.	Cerebral agenesis	0		0.8	0	0	1.8	1.1	0	2.0	46	722	22	5.4	1.2
		20		0.9	0	0	0.6	1.9	0	--	49	714	23	--	1.1
		40		2.7	0	0	0.9	0.2	0	2.0	47	727	24	4.8	1.5
		65		5.7	0	0	2.1	1.8	0	1.1	51	709	30	5.7	1.5
A. L.	Cerebral agenesis	0	3	0.4	0	0	0	0	0	0	44	732	13	5.3	2.0
		20	--	1.3	0	0	--	--	--	0.6	47	732	13	--	1.6
		40	--	1.8	--	--	0	0.4	0	--	48	728	15	5.1	1.8
		65	9	2.2	0	0	0	0.4	0	0.5	52	714	19	5.3	1.8
P. S.	Cerebral agenesis with optic atrophy	0		0.8	0	0	0.9	1.2	0	1.4	51	718	14	4.6	1.8
		20		1.5	--	--	0.2	1.5	0	--	--	---	18	4.5	2.2
		70		3.0	0	0	1.0	1.0	0	1.0	53	717	20	4.6	2.0
M. C.	Cerebral agenesis	0		5.2	0	0	0	0	0	0	52	717	23	5.7	1.5
		40		1.7	0	0	0	0	0	0	59	700	19	5.5	1.5
		60		1.8	0	0	0	0	0	0	58	716	23	5.9	1.6
H. K.	Hemihypertrophy of the body	0	3	0.6	0	0	1.2	0	0	1.0	56	713	11	4.0	0.8
		20	--	0.3	0	0	1.3	0	0	0.4	60	715	10	4.5	0.8
		60		3.2	0	0	0	2.5	0	0.4	77	706	25	4.7	0.8

TABLE I—CONT'D

ENCEPHALOGRAPHY

CASE	DIAGNOSIS	AMOUNT OF AIR INJECTED (C.C.)	CELL COUNT	PHOSPHATASE (UNITS)	TRYPSIN (UNITS)	ANTITRYPSIN (UNITS)	LIPASE (UNITS)	TRIBUTYRINASE (UNITS)	ESTERASE (UNITS)	AMYLASE	SUGAR	CHLORIDES	TOTAL PROTEIN	CALCIUM	PHOSPHORUS
											MG. PER 100 C.C. OF FLUID				
R. P.	Epilepsy	0		0	0	0	0.4	0		2.0	58	713	14	4.5	1.4
		40		1.7	0	0	0.5	0.7	0	--	77	715	20	--	1.3
		60		2.2	0	0	0.4	0.7	0	1.0	56	706	31	5.3	1.4
E. B.	Epilepsy	0	1	0.4	0	0	0	0	0	0.8	54	730	19	5.0	1.3
		90	5	1.5	0	0	0	0	0	0.8	60	729	13	4.9	1.5
M. M.	Mental deficiency	0	3	0.2	0	5	0.3	0	0	0	47	718	13	5.5	1.0
		40		0.3	0	12	0.4	0	0	0	51	723	13	5.8	1.1
D. D.	Microcephalus	0	3	0.7	0	10	0.5	0.4	0	0.4	65	723	18	6.0	1.1
		40	1	12.0	0	0	0.5	9.4	0	0.8	--	---	--	--	1.6
		70		0.9	0	0	0.4	0.4	0	1.6	54	720	21	5.9	1.6

phorus contents of the cerebrospinal fluid. Since it has been found[3] that the enzymatic properties of the cerebrospinal fluid vary in different pathologic conditions of the central nervous system, studies were also made of the trypsin, phosphatase, lipase, tributyrinase, esterase, amylase, and antitrypsin of the cerebrospinal fluid. The results of these studies are reported in this paper in the hope that they will add to the present knowledge of encephalography and offer a broader basis for the interpretation of the effects of that procedure.

METHODS

Specimens of cerebrospinal fluid were obtained from children in whom encephalography was indicated for diagnostic or prognostic purposes. The encephalographies were done by the open method, namely, the removal of small amounts of cerebrospinal fluid and the introduction of a lesser amount of air by means of a syringe.[4] Avertin was used as an anesthetic. In all cases the fluid was withdrawn after an overnight fast, and the studies of the chemical constituents and of the enzymes were started within one or two hours. The fluids included in this study were clear and colorless to begin with and remained so until the end. Occasionally, the last portion of the fluid became slightly turbid, most likely due to the admixture of carbon dioxide from the injected air. We did not include in this study any fluid that contained blood either grossly or microscopically.

Sugar was determined by the method of Somogyi;[5] chlorides by the method of Van Slyke;[6] total protein by the method of Ayer, Dailey, and Fremont-Smith;[7] calcium by the Clark and Collip modification of the Kramer-Tisdall method;[8] phosphorus by the method of Bodansky[9] and nonprotein nitrogen by the method of Koch and McMeekin.[10]

Phosphatase was determined by the method of Bodansky[11] except that 2 c.c. of fluid were used instead of 1 c.c. Trypsin and antitrypsin were determined by a slight modification of the method of Anson and Mirsky;[12] lipase, tributyrinase, and esterase by the method of Crandall and Cherry;[13] amylase by a modification of the method of Ross and Shaw.[14] The details of the methods for the determination of enzymatic activity, as well as a description of the units employed, are given elsewhere.[3]

RESULTS

We shall consider first eleven encephalographies in which determinations were made of the enzymatic activity of the fluids as well as of the simpler chemical constituents. The various constituents will be taken up separately. The results are tabulated in Table I.

Sugar.—In 9 of the 11 cases the sugar content increased during the course of the encephalography. In one case it decreased, and in one case no significant change* was observed. The average sugar content of the first portion of fluid was 53 mg. per cent; that of the last portion studied was 61 mg. per cent.

*By "significant change" is meant a change of
3 mg. per cent in the case of sugar, total protein, and nonprotein nitrogen;
10 mg. per cent in the case of chlorides;
0.3 mg. per cent in the case of calcium;
0.2 mg. per cent in the case of phosphorus.

Total Protein.—In 7 of the 11 cases the total protein content of the fluid increased as cerebrospinal fluid was replaced by air. In one case it decreased, and in 3 instances there was no significant change. The average protein content of the fluid obtained before injection of air was 16 mg. per cent; that of the fluid obtained after the injection of the maximum amount of air was 22 mg. per cent.

Chlorides.—There was no marked variation in the chloride content of the cerebrospinal fluid during encephalography. In 4 instances the chloride value decreased slightly; in 7 there was no significant change. The average value of the chloride content was 722 mg. per cent before injection of air; after injection of air it was 716 mg. per cent.

Calcium.—In 3 instances the calcium was higher in the last portion than in the first. In the other 8 there was no significant change. The average value of the first portion was 5.1 mg. per cent; that of the last portion was 5.4 mg. per cent.

Phosphorus.—In 4 instances the phosphorus content of the fluid increased during the course of the encephalography. In 6 cases no significant changes were noted, and in one case the phosphorus value decreased. The average value of the first portion of fluid was 1.46 mg. per cent; that of the last portion was 1.58 mg. per cent.

Cell Count.—The cellular content of the last portion of fluid was greater than that of the first portion.

Phosphatase.—In 9 cases the phosphatase activity of the final portion of fluid was markedly higher than that of the fluid obtained before injection of air. In 5 of these cases the phosphatase activity increased progressively as the amount of injected air increased. In 2 instances the phosphatase activity decreased slightly after injection of 20 c.c. of air, and then increased when 60 c.c. of air had been injected. In one instance the activity rose sharply after injection of 40 c.c. of air, then decreased after injection of another 20 c.c. In only one instance was the phosphatase activity of the first portion of fluid greater than that of the subsequent portions. In this case (M. C.) the activity of the first portion of fluid was considerably higher than that normally found.[3]

Trypsin and Esterase.—No traces of these enzymes were found. This was to be expected in view of the fact that the fluids of this group were "normal" in the sense that the cellular and chemical constituents were normal. We have shown elsewhere[3] that such fluids rarely, if ever, have trypsin or esterase activity.

Lipase.—Lipase was either absent entirely, or present in small traces in 8 of the 11 cases studied. In 2 cases (M. C. and P. S.) lipolytic activity was present in the first portion of fluid, decreased in the intermediate portions, and returned to the original value in the last portion. Finally, in one instance (H. K.) lipolytic activity was present in the first and second portions of fluid, but not in the last portion. In 10 instances, therefore, there were no significant differences in the lipolytic activity of the first and last portions of fluids; in one case a decrease was found.

TABLE II

VENTRICULOGRAPHY

CASE	DIAGNOSIS	AMOUNT OF AIR INJECTED (C.C.)	CELL COUNT	PHOSPHATASE (UNITS)	TRYPSIN (UNITS)	ANTITRYPSIN (UNITS)	LIPASE (UNITS)	TRIBUTYRINASE (UNITS)	ESTERASE (UNITS)	AMYLASE (UNITS)	SUGAR	CHLORIDES	TOTAL PROTEIN	CALCIUM	PHOSPHORUS
											MG. PER 100 C.C. OF FLUID				
B. S.	Hydrocephalus (severe)	Lumbar fluid		3.1	0	0	0.9	0.7	0	2.6	29	696	103	6.2	1.7
		Ventricular													
		0		0.8	0	6	0.6	1.4	0	3.6	36	654	46	6.1	2.0
		150		0.9	0	6	0.6	1.4	0	3.5	38	674	47	5.6	2.0
		250		0.9	0	6	1.2	2.0	0	2.8	37	701	58	5.6	2.1
		350		0.8	0	17	0.8	2.2	0	2.0	40	682	37	6.1	2.1

Tributyrinase.—Tributyrinase was either absent entirely, or present in small traces in 7 of the 11 cases studied. In 3 instances tributyrinase was present in all the portions of fluid studied. In 2 of these there was no significant difference in activity between the first and last portions; in the other case the last portion had greater activity than the first. In one case (H. K.) the early portions of fluid had no activity, while the final portion had a high tributyrinase value. In 10 instances, therefore, there was no significant difference between the first and last portions; in one case the activity of the last portion was greater than that of the first portion.

Amylase.—Amylase was absent completely, or present in small traces in 5 cases; in 2 other cases amylase was present, but not changed during the course of the encephalography. In 3 instances the amylolytic activity of the last portion of fluid was less than that of the first portion; in one case it was greater than that of the first portion. The changes, however, were small.

Antitrypsin.—Antitrypsin was completely absent in 8 cases. In 2 instances small amounts were present in the first portion of fluid, but not in the later portions. In one case a slight increase was noted in the last portion of fluid. On the whole, the observed changes were not significant.

The results obtained with the 11 fluids of this group can be summed up as follows: The sugar, total protein, and cellular contents of the fluids generally increased during the course of encephalography. The same was true of the phosphatase activity. Occasional increases were found in the calcium and phosphorus contents of the fluids after displacement of cerebrospinal fluid by air. The chlorides decreased slightly in a few cases. No significant changes were observed in the other constituents studied.

In a severe case of hydrocephalus simultaneous lumbar and ventricular punctures were done. Ventricular fluid was then replaced by air, and studies were made of the portions of fluid removed after injection of 150, 250, and 350 c.c. of air.

There was no appreciable difference in the sugar contents of the five portions of fluid. Chlorides were higher in the lumbar fluid than in the ventricular fluid removed before introduction of air. However, after the injection of air, the chlorides rose. Total protein was also higher in the lumbar than in the ventricular fluid. After 250 c.c. of air had been injected, the protein content of the fluid rose, only to fall again on further injection of air. The calcium values of lumbar and ventricular were practically equal. After injection of 150 c.c. of air the calcium value fell, then rose to the original level after 350 c.c. of air had been injected. The phosphorus content of the ventricular fluid was slightly higher than that of the lumbar fluid and was not affected by the displacement of fluid by air (Table II).

Phosphatase activity was higher in the lumbar fluid than in the ventricular. It was not affected by injection of air. Trypsin and esterase were not found in any of the fluid portions. A small amount of antitrypsin was present (6 units). In the last portion of fluid, obtained after 350 c.c. of air had been injected, the antitryptic power was 17 units. Lipase was present

TABLE III

SUGAR, CHLORIDES, NONPROTEIN NITROGEN, TOTAL NITROGEN, AND CALCIUM IN ENCEPHALOGRAPHY FLUID

CASE	DIAGNOSIS	AMOUNT OF AIR INJECTED (C.C.)	SUGAR		CHLORIDES		NONPROTEIN NITROGEN		TOTAL PROTEIN		CALCIUM	
			BEFORE AIR (MG.)	AFTER AIR (MG.)	BEFORE AIR (MG.)	AFTER AIR (MG.)	BEFORE AIR (MG.)	AFTER AIR (MG.)	BEFORE AIR (MG.)	AFTER AIR (MG.)	BEFORE AIR (MG.)	AFTER AIR (MG.)
S. S.	Mental deficiency	60	43	65	710	719	18	17	28	45	4.3	4.8
J. M.	Mental deficiency	100	63	69	723	709	16	19	37	18	4.4	4.3
M. B.	Mental deficiency	70	--	--	723	718	14	14	35	51	5.0	5.4
N. J.	Cerebral agenesis	60	50	60	702	695	10	14	26	21	5.0	5.3
E. S.	Epilepsy	95	61	68	714	717	14	15	15	28	3.9	3.8
M. S.	Epilepsy	75	46	48	707	719	14	14				
J. B.	Epilepsy	80	47	54	756	765	25	31				
P. B.	Epilepsy	120	49	54	638	671	18	18				
G. B.	Epilepsy	90	64	87	685	730	--	--				
M. S.	Epilepsy	135	43	47	684	702	17	17				
S. A.	Epilepsy	100	61	57	720	717	16	16				
M. K.	Little's disease	105	54	78	724	718	13	14				
A. S.	Little's disease	130	50	51	722	723	20	21				
J. M.	Little's disease	130	39	76	711	711	19	19				
S. K.	Little's disease	80	49	47	744	742	28	31				
B. J. S.	Postencephalitic syndrome	80	50	86	749	737	17	21				
S. R.	Postencephalitic syndrome	70	31	40	726	728	14	14				
L. S.	Postencephalitic syndrome	100	47	56	725	725	17	15				
V. W.	Mental deficiency	80	46	58	715	691	12	14				
E. K.	Mental deficiency	80	37	59	737	724	19	19				
B. P.	Mental deficiency	90	59	65	713	716	21	17				
F. A.	Hydrocephalus	200	51	74	707	707	20	20				
W. A.	Microcephalus	60	45	46	691	665	17	17				
R. V. H.	Chronic encephalopathy	160	42	82	723	716	14	14				
D. S.	Lead encephalopathy	170	30	50	737	728	16	16				
	Average values		49	61	716	716	17	18	28	33	4.5	4.7

in varying amounts in all portions of the fluid, but the small quantitative changes observed were without significance. Tributyrinase activity, unlike phosphatase, was greater in the ventricular fluid than in the lumbar fluid. It did not change significantly on injection of air. Amylase was higher in the portion of ventricular fluid obtained before injection of air than in the lumbar fluid. It decreased somewhat on injection of air.

In general, in this ventriculography the greatest contrasts were between the lumbar fluid and the portion of ventricular fluid obtained before the injection of air. The chemical composition of the ventricular fluid was practically unaffected by the injection of air.

We consider next the results obtained in a series of encephalographies in which the effect of displacement of cerebrospinal fluid by air on the sugar, chloride, nonprotein nitrogen, total protein, and calcium contents of the fluid were studied (Table III).

TABLE IV

SUMMARY OF RESULTS

CONSTITUENTS	NO. OF CASES STUDIED	NO. OF CASES INCREASE WAS NOTED AFTER INJECTION OF AIR	NO. OF CASES DECREASE WAS NOTED AFTER INJECTION OF AIR	NO. OF CASES NO SIGNIFICANT CHANGE WAS NOTED	AVERAGE VALUE BEFORE INJECTION OF AIR	AVERAGE VALUE AFTER INJECTION OF AIR
					MG. PER CENT	
Sugar	35	28	2	5	50	61
Chlorides	36	4	10	22	717	716
Nonprotein nitrogen	24	5	1	18	17	18
Total protein	16	10	3	3	20	26
Calcium	16	5	0	11	4.9	5.2
Phosphorus	11	4	1	6	1.42	1.55
Phosphatase	11	10	1	0	----	----
Trypsin	11	0	0	11	----	----
Antitrypsin	11	1	2	8	----	----
Lipase	11	0	1	10	----	----
Tributyrinase	11	1	0	10	----	----
Esterase	11	0	0	11	----	----
Amylase	11	1	3	7	----	----

Sugar.—In 19 of the 24 cases studied the sugar content of the fluid was increased after injection of air. In 4 cases there was no significant change; in one case it decreased. The average value before injection of air was 49 mg. per cent; after injection of air, it was 61 mg. per cent.

Chlorides.—In 4 out of 25 cases studied, the chlorides decreased; in 4 cases they were increased, and in 17 cases there was no significant change. As a whole, neither the decrease nor the increase was very significant. The average value before injection of air was 716 mg. per cent; after injection of air it was still the same.

Nonprotein Nitrogen.—Of 24 cases in which the nonprotein nitrogen content was determined before and after the injection of air, no significant change was found in 18. In one instance the value decreased; in 5 it increased. The average value before injection of air was 17 mg.; after injection it was 18 mg. per cent.

Total Protein.—In the 5 cases studied for the protein content, the total protein was increased in 3 and decreased in 2. The average before injection of air was 28; after injection of air it was 28 mg. per cent.

Calcium.—In 3 cases the calcium content increased slightly on injection of air; in the other 2, no significant change was noted. The average calcium values of the first and last portions of fluid were 4.5 and 4.7 mg. per cent, respectively.

Cells.—The cellular content of the last portion of fluid was greater than that of the first portion.

The results of the entire study are summarized in Table IV. In general, sugar, total protein, and phosphatase, as well as cellular content, rose during the course of encephalography. Calcium and phosphorus showed a slight tendency to rise. Nonprotein nitrogen, chlorides, antitrypsin, trypsin, lipase, tributyrinase, amylase, and esterase did not change significantly when cerebrospinal fluid was displaced by air.

DISCUSSION

What is the cause of the changes observed? There are two possible explanations for the rise in the sugar content of the fluid. One is in terms of the difference in location of the fluid making up the various portions withdrawn during encephalography. The first portion of fluid removed is lumbar fluid. Since it is known that the sugar content of cisternal fluid is slightly greater[15] and that of the ventricular fluid considerably greater[16] than that of lumbar fluid, it follows that the higher sugar content of fluid obtained after injection of air may be due in part to its being ventricular fluid. However, this explanation is not satisfactory for the other chemical changes observed during encephalography. Ordinary cisternal or ventricular fluid is generally lower in cellular and protein contents than lumbar fluid.[16, 17] This is not the case in encephalography where the latter portions of fluid contain more cells and protein than the first portion. A second explanation for the increase in sugar is in terms of the hyperglycemia which has been found in many cases of encephalography.[18-20] Such hyperglycemia, either alone or in conjunction with the increased meningeal permeability associated with an aseptic meningeal reaction,[21] would tend to increase the cerebrospinal fluid sugar.

The increase in cells and protein may also be due to an aseptic meningeal reaction caused by the presence of air in the ventricles and subarachnoid space. This hypothesis is supported by the fact that in aseptic meningitis induced experimentally by intraspinal insufflation of air,[22] a marked pleocytosis results, together with an increase in total protein. On the other hand, Riser and Planques[23] have found increased cellular and protein contents during the course of continued simple spinal drainage. This seems to indicate that the chemical changes in the cerebrospinal fluid during encephalography are due in part to the presence of air and in part to the readjustment of the cerebrospinal system to loss of fluids.

The importance of an aseptic meningeal reaction is further indicated by the tendency of calcium to rise during encephalography. In view of the very low

calcium content of brain tissue[24] and the increased calcium content of the cerebrospinal fluid often found in meningitis,[25] the most likely source of calcium increase during encephalography is increased meningeal permeability. In those instances where the calcium values rose considerably during the procedure the total protein content also rose sharply (see Table I: R. P., H. K.; Table III: M. B., S. S.). The combination of increased calcium and total protein may be an indication of meningeal or chorioidal irritation. In some cases, calcium, sugar, and total protein were hardly affected during encephalography. This may point to a very slight meningeal reaction. However, in a few cases the sugar content remained practically constant while a marked cellular reaction took place (see Table III: A. S., M. S., W. A., and S. A.). Unfortunately, studies of the calcium and total protein contents were not made in these instances. The subject of an aseptic meningeal reaction in encephalography requires further investigation, although at present it seems to be the simplest hypothesis which can be offered for the chemical changes in the cerebrospinal fluid during encephalography.

Probably the most interesting finding reported in the present paper is the phosphatase activity of the fluid, which seemed to parallel the amount of cerebrospinal fluid removed. Since the blood has considerable phosphatase activity, it seems possible that some phosphatase may enter the cerebrospinal fluid because of the increased meningeal permeability caused by irritation of the meninges and chorioid plexus. It is difficult to explain, however, why other enzymes, such as lipase, tributyrinase, and amylase, as well as antitrypsin, would not also enter the cerebrospinal fluid. It would be necessary to hypothesize different barrier permeabilities for the different enzymes. There are at present no adequate experimental data on which to base such an hypothesis. We have found[26] that in infectious meningitis some enzymes probably do cross the meningeal barrier, but no quantitative relationships have been found between the degree of involvement of the meninges and the degree of permeability of the meninges to enzymes.

Another possibility is that phosphatase from brain tissue reaches the subarachnoid space via the perivascular spaces in the course of the adjustment of the cerebrospinal fluid to the presence of air.

The importance of effects, such as those discussed above, is perhaps more strongly indicated by recent work on simple and forced drainage of cerebrospinal fluid. Kubie and Retan[27-32] have studied at great length the physiologic and clinical effects of spinal drainage coincident with the intravenous injection of hypotonic saline. They often found an increase in the cell count of the fluid during forced drainage, and are of the opinion that this is due to drainage of the perivascular channels. Retan has used "forced perivascular drainage" in the treatment of diseases of the central nervous system, such as meningitis, encephalitis, poliomyelitis, syphilis, and chorea. Fay[33] has suggested that the beneficial results obtained by Kubie and Retan were due to the increased blood volume of the brain caused by removal of cerebrospinal fluid, rather than to perivascular drainage. He considered that encephalography produces a forced drainage in that all the cerebrospinal fluid may be readjusted when an appro-

priate amount of air has been introduced to take its place. This possibility is supported by the work of Riser and Planques,[23] who found, in an experimental study of simple and forced drainage in dogs, that the number of cells and the protein content of the fluid increased without change in the chlorides during the course of drainage. Histologic examination of the brain and spinal cord of the dogs showed a mild lymphocytosis in the arachnoid meshes, but no active cellular proliferation in the perivascular spaces of the brain and spinal cord. Riser and Planques concluded that the increases in lymphocytes and protein are merely reactions of the chorioid plexus and meningeal vessels, and do not indicate drainage of the neural parenchyma itself.

Since the changes observed during encephalography, simple drainage, and forced drainage are similar with respect to those constituents of the cerebrospinal fluid which have been studied, it seems reasonable to assume that the three processes have some factor in common. This factor may be the adjustment of the cerebrospinal fluid to changes in intracranial hydrodynamics caused by the removal of cerebrospinal fluid. In encephalography the adjustment may be complicated by an aseptic meningeal reaction due to the presence of air. In forced drainage the intravenous injection of hypotonic saline may be a complicating factor, but this is not borne out by the chemical studies which have so far been made. Further experimental studies on forced drainage, emphasizing possible changes in the enzymatic activity, calcium, and phosphorus, may aid in the solution of this problem.

CONCLUSIONS

1. The chemical constituents of the cerebrospinal fluid were studied during the course of encephalography. The constituents investigated were sugar, chlorides, nonprotein nitrogen, total protein, calcium, phosphorus, phosphatase, lipase, tributyrinase, esterase, amylase, and antitrypsin.

2. The phosphatase, sugar, and total protein contents of the cerebrospinal fluid are increased after injection of air into the spine and ventricles.

3. Calcium and phosphorus increased in some cases, but less generally than phosphatase, sugar, and total protein.

4. Chlorides, nonprotein nitrogen, lipase, tributyrinase, esterase, amylase, and antitrypsin underwent no consistent significant changes.

5. The changes observed are most probably due to an aseptic meningeal reaction caused by the presence of air in the ventricles and subarachnoid spaces. Similar changes, however, have been reported during simple and forced drainage of cerebrospinal fluid.

6. The increase in sugar content may also be due in part to the fact that the later portions of encephalography fluid are ventricular rather than lumbar or cisternal.

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HEAT LABILE FACTORS NECESSARY FOR THE PROPER GROWTH AND DEVELOPMENT OF CATS*

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THIS report covers a three-year study of the effect of raw and cooked foods upon cats. The mother cats were placed on special foods four to six months before breeding. The diet of cats given raw food consisted of raw meat and dry feed raw milk; the diet of cats given cooked food consisted of cooked meat and dry feed raw milk. Other studies have shown that the properties of dry feed raw milk are similar to those of pasteurized milk. Both groups were given a liberal amount of cod-liver oil to insure an adequate supply of vitamins A and D. The study deals chiefly with the offspring produced from these mother cats.

All of the cats used in this experiment were kept in large outdoor pens. The sides and tops of the pens were screened with chicken wire only, so that the cats had the benefit of all the sunshine available. Each pen was divided into two portions—a yard 4 feet wide and 12 feet long, with a 7 foot ceiling, and a roofed section 6 feet square with a 6 foot ceiling, a wooden floor and bedding. The floor of the pen was bare earth covered with fine sand.

During this three-year period, 63 living kittens were born of parents fed raw food, with an average weight of 119 gm. at birth, and 47 kittens were born of parents fed cooked food, with an average weight of 100 gm. There were 4 dead kittens born in the raw-food group and 16 in the cooked-food group. An example of the effect of the diet of the mother upon the birth weight of the offspring is the following: Cat H. F. fed a cooked-food diet produced a litter of 4 kittens, whose average weight was 77 gm. She was then placed on a raw-food diet, and her litter for the next year consisted of 5 kittens, with an average birth weight of 116 gm., while the following year (still on a raw-food diet) the litter consisted of 3 kittens, with an average weight of 137 gm. The opposite result may be produced by taking a mother cat who has been on raw food and placing her on cooked food.

Cat D given a cooked-food diet produced a litter of 5 kittens, with an average weight of 105 gm., and the following year her litter consisted of 6 kittens, with an average weight of 91 gm.

If the mother cat is kept on cooked food for more than two years, she usually dies during delivery.

The cats fed cooked food occasionally produce a premature or full-term litter of stillborn kittens. In several instances mother cats on a cooked-food diet died during delivery. One cat was unable to deliver her kittens even though in labor for seventy-two hours. Delivery complications such as these have not been found in the case of cats placed on a raw-food diet.

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The cats fed cooked food were smaller in build; though the bones of their legs were smaller in diameter, they were frequently longer and their fur development was much poorer than that of the cats fed raw foods. Muscle tone and visceral development of cats fed raw meat was much greater than that of cats fed cooked meat. Disturbance in the genital development and descent of the testes was common in male kittens of mother cats on cooked food. Cats on cooked-food diet were of a much more nervous disposition than cats on raw-food diet; they paced the pens, they were not very friendly, and they were often quite vicious.

One of the first defects noticed in the cats fed cooked food was poor dental development. The temporary teeth of both the cats fed cooked foods and those fed raw foods seemed well developed. However, when the permanent incisors displaced the temporary ones, the cats on the cooked-food diet usually developed three or four irregularly spaced, uneven, crowded incisors instead of the usual six. This was true of both the upper and lower jaws. Although a few of the cats on the raw-food diet had this same condition, it occurred from three to four times as often in the cats on the cooked-food diet as in those on raw food. This condition became even more pronounced in the second and third generation of cats on the cooked-food regime.

The femurs of these animals were removed, and the entire bone analyzed for its calcium and phosphorus content. The kittens of both the mother cats who had been fed raw food and those who had been fed cooked food had approximately the same amount of calcium and phosphorus at birth; quite often this was within 2 or 3 per cent of that found in the femur of the mother cat. After the first two weeks a marked depletion of the calcium and phosphorus of the bone occurred. This no doubt corresponds with the period of greatest growth. Two months later the bones of the kittens on the raw-food ration were gradually approaching normal in respect to these salts while those of the kittens on the cooked-meat ration still lagged behind. This effect was still more pronounced in the second and third generations; that is, the bones of second and third generation kittens given cooked food, even after they reached adult life, were markedly deficient in calcium and phosphorus content. Corresponding litters of kittens fed raw food had from two to three times as much calcium and phosphorus in their bones. If the first, second, and third generations were kept on a cooked-food diet, the third generation kittens were in such poor condition that they lived at the longest only two months. We were unable to carry our experiments beyond the third generation on the cooked-food diet, because no cats survived beyond that time.

Our experience has been that once a deficiency is produced in kittens it cannot be reversed even under intense therapy. A well-developed cat can be maintained in a healthy state on deficient diets if thyroid and adrenal hormones are added to the dietary. A deficient kitten, however, even if given raw food, thyroid and adrenal hormones, insofar as we have seen, does not become a normal cat.

If a mother cat has been on a cooked-food diet from twelve to eighteen months, it takes at least three years on a raw-food diet before her kittens begin to approach normal.

Upon the death of the cats a complete post-mortem examination was made. A study of microscopic sections of the lungs of second and third generation cats showed that cooked food was responsible for the production of certain respiratory conditions. The lungs of these cats showed hyperemia, some edema, and partial atelectasis, while those most deficient showed bronchitis and pneumonitis. The kittens fed raw food had normal lungs, although occasionally a cat was found whose lungs showed a mild hyperemia.

Five of the cats given cooked food showed suppurative inflammation of the thigh. Smears of the pus showed the presence of streptococci and staphylococci. The femurs were fragile and showed marked osteoporosis, with marked thinning of the cortex. In 2 of these 5 cats the bone had been partially eaten away and only chips and fragments remained.

In several cases we found a hypothyroid condition among the cats fed cooked food. The thyroids showed scanty colloid, and the acini were small. This was not observed in the thyroids of cats given raw food.

The ovaries of the cats fed cooked food showed, as a rule, only a few developing follicles and primordial ova, while those cats fed raw food showed active oogenesis with normal and large numbers of developing follicles. In males the testes of the cats fed cooked food showed a lessened number of spermatozoa and many of them showed an absence of spermatozoa but with active spermatogonia. Cats fed raw food showed active spermatogenesis.

STUDIES IN BLOOD PRESERVATION*

Fate of Cellular Elements in Relation to Potassium Diffusion

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IN A PREVIOUS publication[1] we reported a tenfold rise in plasma potassium of preserved blood. This was not due to bacterial contamination. It was increased by trauma, such as shaking, and was definitely modified by the area of the interface between the sedimented cells and the overlying plasma.

The question was raised as to whether this increase in plasma potassium was a pure diffusion process or whether it was due to actual cell destruction. This aspect of the problem is reported here.

METHOD

Five cubic centimeters of freely flowing venous blood were collected in each of 35 sterile round-bottomed test tubes containing 5.0 mg. of heparin as an

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anticoagulant. After inverting each tube three times, they were plugged with cotton and kept in a refrigerator at approximately 4° C. throughout the period of the experiment.

Each day one tube was taken from the refrigerator, and centrifuged for one hour. After 0.5 c.c. of plasma had been removed for potassium analysis,[2] and after the blood had been thoroughly mixed by inverting the tube fifteen times, the following determinations were made:

(1) Red cell count.
(2) Hemoglobin (Hellige).
(3) White cell count.
(4) Differential white cell count.
(5) Platelet count.
(6) Mean cell diameter of red blood cells (Halometer method).

RESULTS

The donor, A. J., was a professional man of International Group 0.

	Venous Blood		
	At start of phlebotomy	At end of phlebotomy (590 c.c.)	
Hematocrit	46.0	44.7	per cent cells
Plasma specific gravity	1.0286	1.0266	
Plasma proteins	7.38	6.70	gm. per cent
Whole blood potassium	212.0		mg. per cent
Plasma potassium	21.5		mg. per cent
Cell potassium (calculated)	434.0		mg. per cent
pH		7.51	
Values on Initial 5.0 c.c. Sample*			
Red blood cell count		5,500,000.0	
Hemoglobin		14.2	gm. per cent
White blood count		7,000.0	
Differential white cell count			
Polymorphonuclear leucocytes		52.0	per cent
Eosinophilic leucocytes		2.0	per cent
Basophilic leucocytes		1.0	per cent
Lymphocytes		42.0	per cent
Monocytes		3.0	per cent
Platelets		140,000.0	
Mean cell diameter		7.4	
Plasma potassium		20.6	mg. per cent

The results are graphically represented in Charts 1 to 4.

*Corrected for 0.5 c.c. plasma removed.

DISCUSSION

Red Blood Cells.—The maintenance of the red blood cell counts at approximately a constant level over a period of a month suggests that, in the steady increase of the potassium content of the plasma, cell destruction plays at most only a small part.

Durán Jordá and his co-workers[3] reported there was a loss of 1,500,000 erythrocytes over a period of sixteen to twenty days in blood conserved in citrate. They suggested the stroma of these destroyed cells might play a part in some of the slight posttransfusion reactions. We have not found changes as

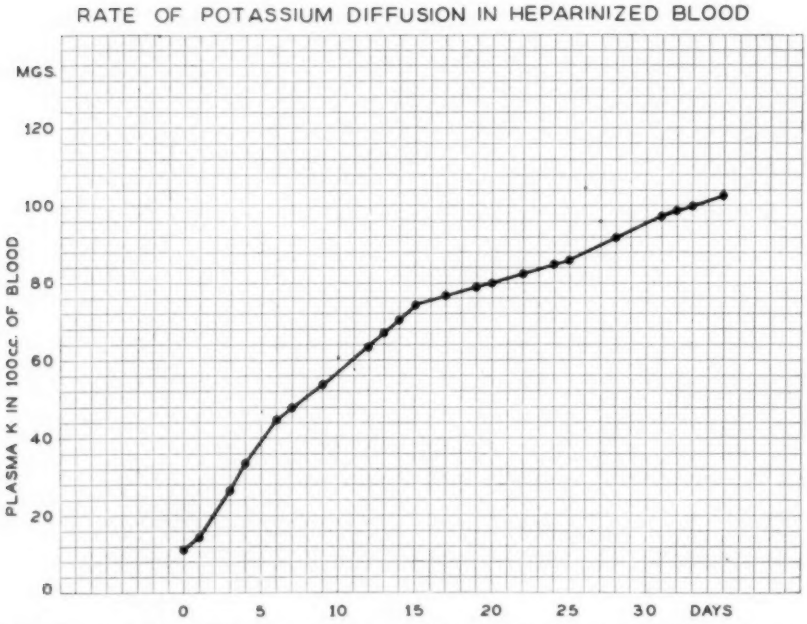

Chart 1.—Potassium diffusion in heparinized blood. Each point on the chart represents a determination done on different test tube samples each day and is calculated to represent the actual amount of potassium in milligrams in the plasma of 100 c.c. of blood. The values are approximately the same as in previous experiments where daily determinations were done on the same blood with the final curve representing a summation of the increments.

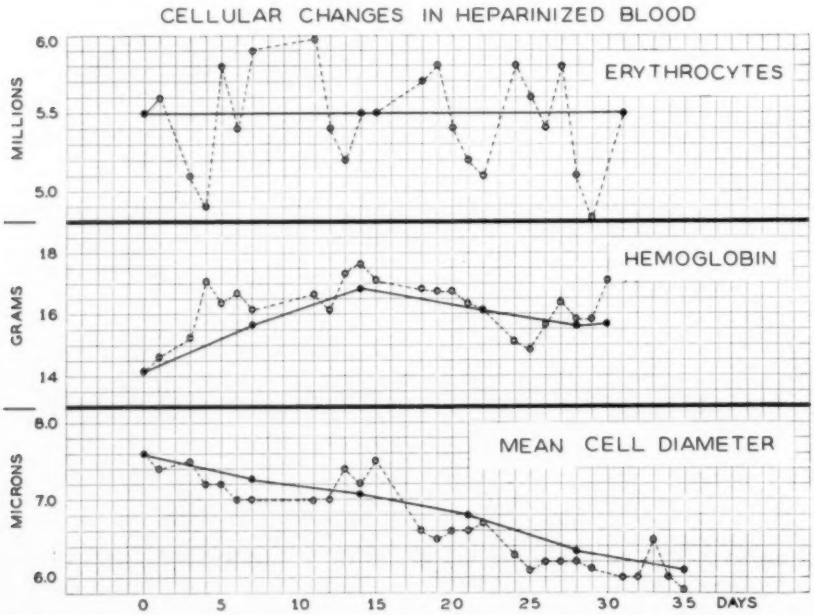

Chart 2.—*Upper third.* Red cell counts varied between 6,200,000 and 4,800,000, the mean being 5,500,000. No actual loss in number of red blood cells.

Middle third. Hemoglobin values varied between 14.2 and 17.6 gm. per cent. The curve, allowing for slight concentration due to evaporation, represents a constant hemoglobin content. The drop after the fifteenth day may be accounted for by the gradually increasing amounts of hemogrobin removed in the plasma taken for potassium determinations.

Lower third. There was a gradual decrease in the size of the red blood cells from an initial 7.6 microns to a final 5.8 microns as the cells lost both hemoglobin and potassium.

great as they report. The fact that all of our tubes were centrifuged for an hour before each count may play some part in this discrepancy.

Yudin,[4] from Russia, has reported that there is no loss in ability of cells so stored to carry oxygen, hence the chief biological function of the red blood cells seems unimpaired.

Hemoglobin.—The hemoglobin content remains constant. This confirms the findings of previous writers. The work of Amberson[5] has indicated that the part of the hemoglobin which escapes from the cells to give the usual picture of hemolysis is still capable of functioning normally.

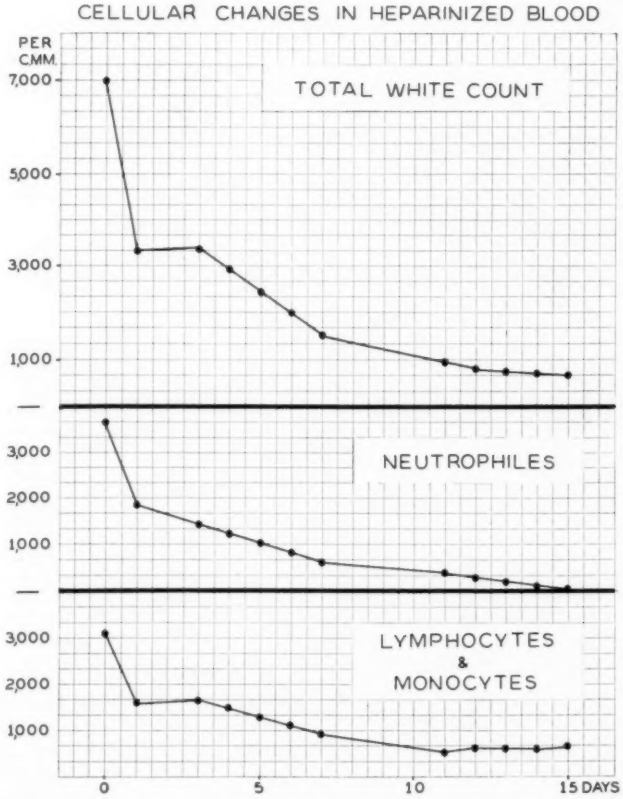

Chart 3.—*Upper third.* The total white blood cell count fell 50 per cent in twenty-four hours, the cells lost shape and gradually disappeared by the sixteenth day; those remaining appearing only as gelatinous-like clumps. Range 7,000 to 675 cells.

Middle third. The polymorphonuclear leucocytes showed earliest and most rapid changes; their nuclei soon losing shape and disintegrating. Range, 3,600 to 14 cells.

Lower third. Lymphocytes and monocytes disappeared at a slower rate and retained their shape, size, and staining better than the leucocytes, being quite distinct when found on the thirtieth day. Basophilic and eosinophilic cells, while too few to be charted, retained their shape, size, and staining qualities. The latter were least changed and very distinct on the thirtieth day. Range, 3,100 to 654 cells.

Volume Index and Diameter of Red Blood Cells.—The volume index of the cells decreases in successive specimens. There is a diminution in white blood cells, but the greater part of the decrease seems due to the diminution of the size of the red blood cells. At the end of thirty days these cells may lose as much as 25 per cent of their chief base, 20 per cent of their hemoglobin, and decrease 20 per cent in diameter. Ponder[6] has shown that red blood cells do not

always act as perfect osmometers, a fact which he attributes to the possible loss of their salt content.

White Blood Cells.—The total white blood cell count rapidly diminishes with the polymorphonuclear leucocytes disintegrating most rapidly. The reported loss of half of the complement of the blood by the sixth or seventh day, and complete destruction in fifteen to twenty days, may be associated with this loss of leucocytes.[3]

The lymphocytes are more resistant. At the end of thirty days they are easily recognizable when found, but there are so few that counts are uncertain. This holds true, likewise, for monocytes.

The eosinophiles appear most resistant. The nucleus breaks up late and the granules remain unusually well defined.

Durán Jordá[3] has reported similar findings.

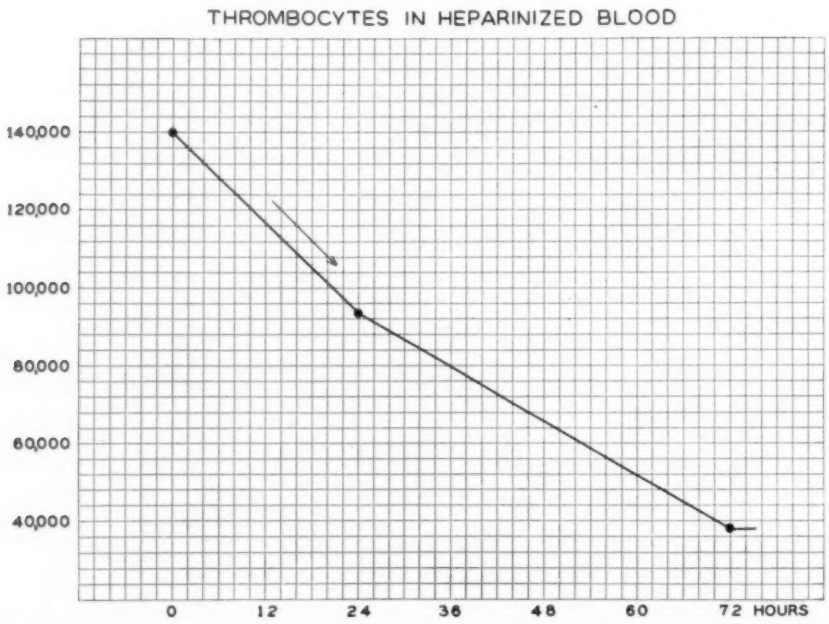

Chart 4.—Thrombocytes rapidly fell to about 40,000 and for thirty days remained approximately at this figure. Only the first three days are charted.

Thrombocytes.—The platelets in the initial venous blood sample numbered 140,000. These rapidly diminished in the first three days and remained at about 30,000 for fifteen days. Centrifugation may have some part in these counts. This suggests, however, that syndromes presenting thrombocytopenia would not be benefited by blood which had been stored too long.

Potassium.—The rate of potassium loss from the cells in the individual test tubes is of the same order as that found in the flasks from which daily samples were taken. The potassium content of white blood cells (those obtained from the buffy coat in case of lymphatic leucemia) is greater than the red blood cells. In normal blood their relative number is insignificant. However, their early rapid destruction may account in part for the steep slope of the curve during the first few days.

CONCLUSIONS

1. In heparinized preserved blood there is little or no actual loss in the number of red blood cells over a period of thirty days.

2. The hemoglobin content remains constant in the total sample, though 15 to 25 per cent may be found in the plasma.

3. The mean cell diameter of the red blood cells is reduced approximately 20 per cent in thirty days.

4. The polymorphonuclear leucocytes are diminished by 50 per cent in forty-eight hours and are amorphous masses in fifteen days.

5. The lymphocytes, monocytes, and eosinophiles do not disintegrate so rapidly, the latter are particularly well preserved.

6. The thrombocytes rapidly fall to a low level, then remain constant at about 30,000 for about fifteen days.

7. Potassium in the plasma of heparinized blood reaches a level ten times normal in thirty days.

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GLOMUS TUMOR OF ARM*

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TO DATE 106 cases of glomus tumor have been reported in the literature. It is our purpose to add a case in which the tumor occurred at the site of a pre-existent "blue birthmark" and whose onset showed a suggestive relationship to trauma.

The present concept of the glomus is based upon the work of Sucquet, Hoyer, Grosser, Schumacher, Masson, Clara, Popoff, and others. A glomus unit includes the following components: (a) an afferent artery, (b) Sucquet-Hoyer canal, (c) neuroreticular and vascular structure around the Sucquet-Hoyer canal, (d) an outer lamellated collagenous tissue, and (e) a primary collecting vein. This unit comprises the arteriovenous anastomosis.

The most constant sites of the normal glomus are in the hands and feet. Here they may be found in the ventral surfaces of the hands and feet, in the

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region of the nail beds, in the tips of the digits, in the palmar surfaces of the first, second, and third phalanges, and in the thenar and hypothenar eminences of the hand, and in the sole near the heel. Investigation has also disclosed them to be present within the bones of the terminal phalanges, the skin, lips, nose, eyelids, tip of tongue, tendons, gums, pia mater, dura, iris, ciliary bodies and endocardium. These glomic units are found in a definite zone of the corium, just below the web of subpapillary arteries and veins.

Popoff was unable to find them in fetuses aged 4½ months to term. Their development occurred during the first two months of postnatal life. He also demonstrated that over the age of 60 there is a diminution in the number of Sucquet-Hoyer canals per square centimeter. This decrease is progressive with increasing age. They are absent in supernumerary digits. Grosser was unable to find them in reptiles, but he did find them in many mammals.

The ascribed function of the glomus body is to contribute to the control of the temperature-regulating mechanism of the body and to coordinate with the mechanism of peripheral vascular pressure control. When there is exposure to cold, the glomic system maintains and even raises local temperature. This is accomplished by diverting blood from the capillaries into collecting veins which have a highly developed surface area. When the glomic system is fully opened, it aids the dispersal of heat by allowing an enormous flow of blood to pass through the digits. When closed, it reduces the dispersal of heat. With an elevation of peripheral pressure it is capable of diverting arterial blood and thereby relieving the arterial system of a part of its burden. The absence of the glomic system during fetal life and its somewhat tardy development postnatally may be contributory to poor control of body temperature in premature infants and some newborns.

The most common aberration reported clinically is tumor formation. As such it occurs in sites where glomic bodies are normally found, the extremities being the most common sites. Although it is usually a single tumor, two cases of multiple tumors have been described by Bergstrand. Radasch found four other reports of multiple tumors. The age occurrence is between 20 to 80. One case has been reported in a child of 6. The shortest duration of all the tumors was six weeks, the longest was 37 years. Subungual glomal tumors usually were noted at an average age of 25. Tumors at other sites were noted at an average age of 41. The outstanding clinical symptom was pain, intermittent or constant. Weidman and Wise have, however, reported a case in which they found multiple "glomic tumors" which were painless. Histologically their case showed chiefly cavernous dilatation and no nerve involvement. They considered their tumors to be a microscopic varicosity of a glomus rather than a true tumor.

Most of the tumors revealed no clinical evidence of any etiologic factor. Several observers were able to elicit a suggestive relationship to trauma. Slepyan reported finding two such cases. Jirka and Scuderi elicited a history of trauma. Our case gives a history of trauma.

The glomal tumor has been accredited benign, and to date no glomal tumor occurring at one of the usual sites has been reported otherwise. Stout, report-

ing a series of 11 cases, noted a recurrence at the original site eight years after removal. Recently, Kirshman and Teitelman, reporting a fatal metastasizing tumor of the omentum, described a histopathologic picture simulating a glomangioma. Since no normal glomic bodies have ever been described in the omentum, they raise the question of origin.

All of the elements of the Sucquet-Hoyer canal and its surrounding components have been designated as being the origin of the tumors cells.

Jirka and Scuderi implied an endothelial origin to the tumor cells. Krompecher considers them as angioblasts. He was unable to demonstrate myofibrils. Geschickter, because of the method of termination of the neurofibrils, believed the glomal cells to be modified nerve cells. Popoff, in his study of normal glomus, found amid the ordinary muscle cells, large epithelioid cells with clear cytoplasm and round or globular nuclei. Followed in serial section, these epithelioid-like cells appeared to be transversely cut muscle cells with voluminous cytoplasm and slightly elongated polyhedral nuclei.

Popoff, further in his study of the digital vascular system, had noted primary pathologic changes of the glomic unit in nonspecific cellulitis, senile arteriosclerotic gangrene of the foot, and diabetic gangrene. In thromboangiitis obliterans the pathologic changes were secondary.

The patient, a white male, aged 42 years, complained of a painful nodule on the left arm. He stated that a small nodule had been present above the elbow for ten years as a "blue birthmark." It had never been painful. Eight months prior to admission, while putting his arm out of an open automobile window, he struck the region of the nodule against the frame. At that time he states that it felt like a nail had been stuck into his arm. From then on he had a constant dull ache at the site of the injury. About two months after the injury he began to experience episodes of intermittent pain at the site of the nodule. These pains he described as rapidly succeeding paroxysms "as though a needle was stuck into his arm." Each episode was associated with a similar pain, located at the level of the left sixth rib at the anterior axillary line. The nodule was painful when pressed. He noted no increase in size. Local examination revealed a small blue area, ¼ inch in diameter, on the posterior aspect of the left upper arm, 8.5 cm. above the olecranon. The nodule, although sensitive to pressure, revealed no "trigger tenderness." The remainder of the physical examination, except for several small scattered hemangiomas on the anterior chest wall, was of no significance. The blood Wassermann and Kahn tests were negative.

Gross: The specimen consisted of an oval piece of skin, 1.5 cm. in long diameter, covering a small amount of subcutaneous tissue. In this tissue was found a small, lentil-shaped, dark brown, sharply circumscribed nodule.

Low-power examination revealed a nodule bordered by a well-defined fibroelastic capsule. The nodule was formed by intercommunicating vessels. Each vessel was surrounded by a mantle of spheroidal cells. These units were separated by small areas of collagenous tissue. Under higher magnification the endothelium of the vessels consisted of elongated cells resting on a collagenous membrane which varied in thickness. With the van Gieson's stain the collagenous fibrils were noted to radiate from the vessels and showed a dendritic proliferation between the tumor cells. Stained by the Bielschowsky-Maresch method, the connective tissue fibrils were deep black on a clear background. Thick collagen bundles were reddish brown.

The cells were in single and in group arrangement and appeared related to the collagenous fibrils as the fruits of a laden-down tree to its branches. The tumor cells about the vessels were distributed in fantastic waves in a more or less pyriform arrangement. The cytoplasm was ill defined and faintly staining. From it projected slightly elongated processes. The nuclei were stained deeply and contained granular chromatin material. No mitotic figures

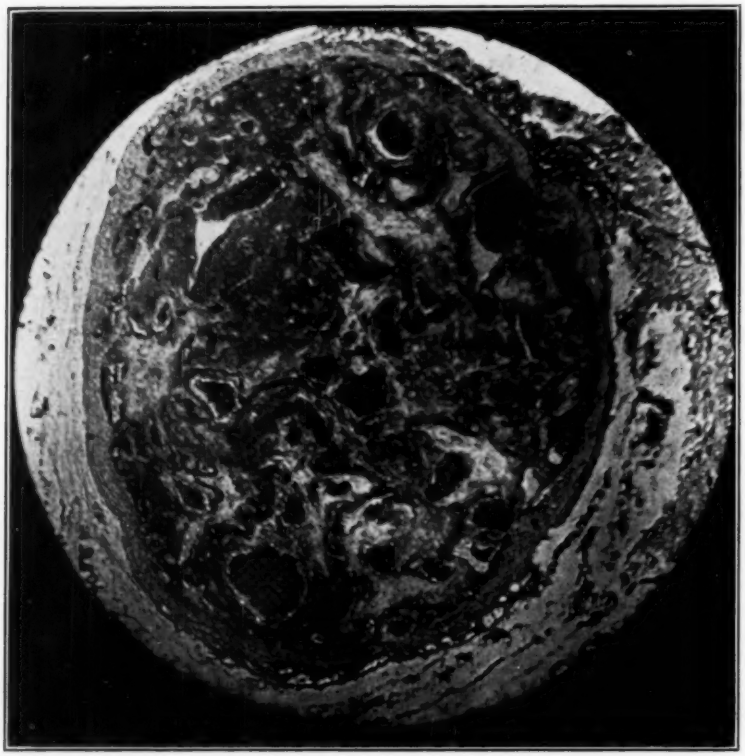

Fig. 1.—Low power.

Fig. 2.—Medium power.

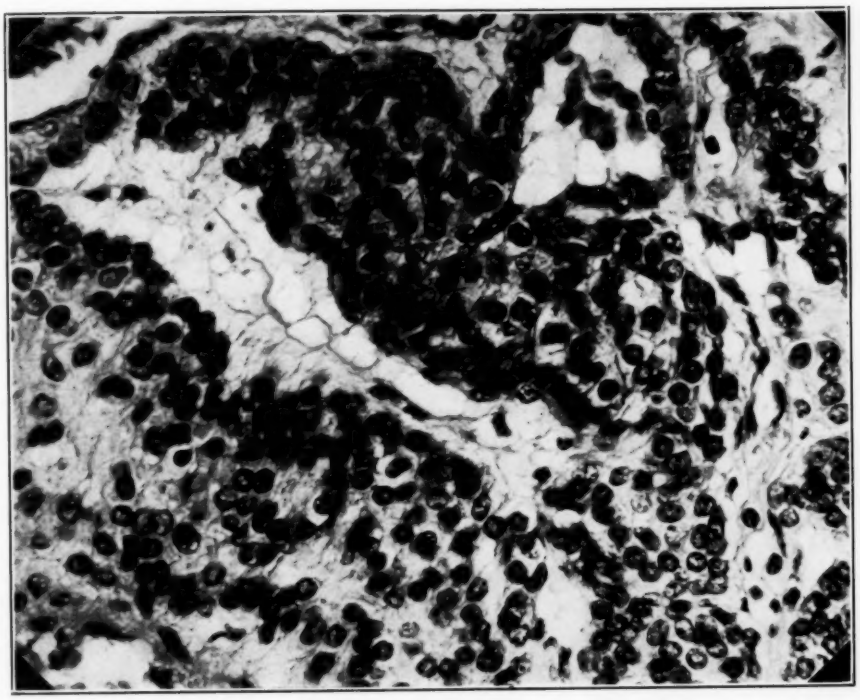

Fig. 3.—High power.

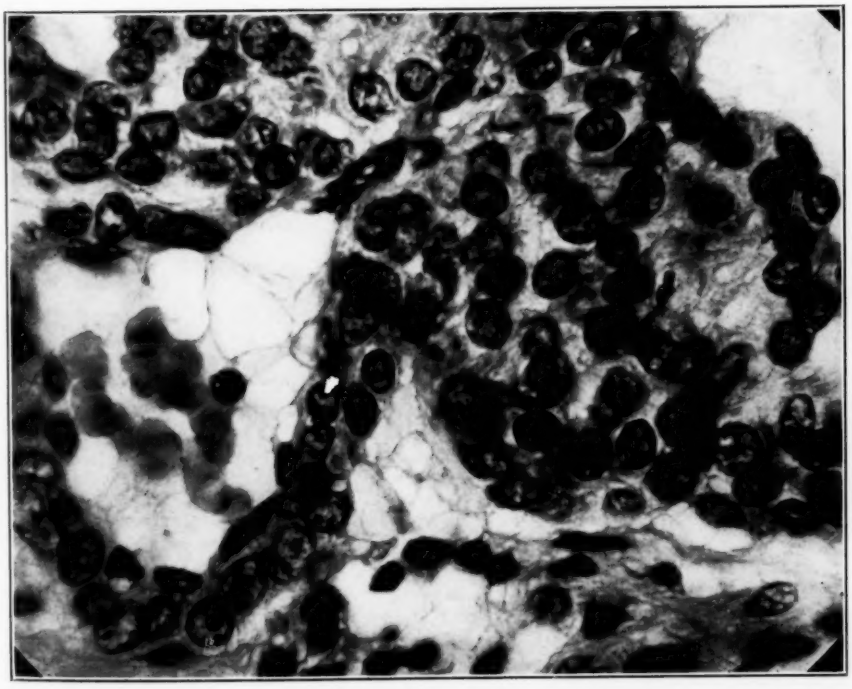

Fig. 4.—Oil immersion.

were noted. Sections stained by Bodian's method disclosed the presence of nonmyelinated nerve fibers which appeared to be in continuation with some of the foregoing cellular processes. Between the tumor cells in the collagenous tissue occasional fibroblasts and fibrocytes could be seen.

In accordance with other observers and with the classical work of Masson, we believe that the tumor cells originate from the muscular elements of the Sucquet-Hoyer canal and that they are classified as myoblasts. We further believe that these cells are connected to the periglomal nerve elements by nonmyelinated nerve fibers.

SUMMARY

A case of glomus tumor occurring in a 42-year-old white male is reported. The tumor occurred at the site of a previous "blue birthmark," following injury to that site. The "birthmark" was of ten years' duration, and the glomus tumor was of eight months' duration. The tumor cells appear to be of myoblastic origin. We suggest that there exists a connection of these cells to the periglomal nerves by means of nonmyelinated nerve fibers. In view of the fact that the exact nature of the previous "blue birthmark" is unknown, it is difficult to postulate a relationship of the glomus tumor to it, although some relationship is extremely suggestive. We believe that the glomus tumor is a type of angioma, a complex type, in which the neuromyoblastic elements play an important role for clinical manifestations and for histologic morphology.

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EFFECTS OF INTRAPERITONEAL INJECTIONS OF STAPHYLOCOCCUS ANTITOXIN ON SUBCUTANEOUS STAPHYLOCOCCIC INFECTION IN MICE*

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THE mechanism of staphylococcus immunity, both local and general, is not clearly understood. Much emphasis, however, has been placed upon antibacterial immunity. Wright and Douglas[1] thought that opsonins played the important role in local immunity. It is now well known that staphylococcus vaccines produce an increase in the opsonic index, and the animals show a greater resistance to staphylococcus infections. Cannon and Pacheco,[2] in their study on immunity, immunized guinea pigs with a staphylococcus vaccine and then injected a virulent culture of the same bacterium subcutaneously. Histologic sections of the skin of these animals showed that the organisms formed clumps and clusters. A greater number of leucocytes and more phagocytosis were present in the sections from the immune pigs than in the normal controls.

Recently attention has been directed toward antitoxic immunity in staphylococcus infections.[3-5] The specific antitoxin does protect rabbits against a lethal dose of staphylococci. This protection is thought to be the result of a neutralization in vivo of the toxin by the antitoxin.[6] Investigators, in studying this immunity, have been concerned primarily in obtaining complete protection against the lethal action of cultures of staphylococci following an intravenous injection. Little attention has been given to the effects of staphylococcus antitoxin on local staphylococcus infections.

The present study was made to determine the type of reaction that would occur in the subcutaneous tissues when toxin- and nontoxin-producing strains of staphylococci were injected locally in normal mice and mice passively immunized with staphylococcus antitoxin. Staphylococcus toxin was also injected into a group of the normal mice to compare the reaction produced by the toxin with that produced by the bacterial cells.

MATERIALS AND METHODS

The staphylococcus toxin† used in this study contained at the time of its preparation 8,000 dermonecrotic units per cubic centimeter. It was diluted 1:100 with physiologic saline immediately preceding the inoculation. Injections of 0.05 c.c. of this diluted toxin were made subcutaneously into each side of the abdomen of 20 white mice.

The toxin-producing strain was an *aureus* variant from a culture obtained in May, 1933, from the pharynx of a patient with agranulocytic angina. The

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†The toxin was supplied through the courtesy of Lederle Laboratories.

lethal effect of this variant on rabbits was recently reported.[6] The ability to produce toxin was determined by the method of Biggers.[8] The supernatant fluid obtained from washing a forty-eight-hour growth from an agar slant (15 by 2.5 cm.) contained enough hemotoxin in 0.05 c.c. to lyze completely 2 c.c. of a 1 per cent suspension of rabbit red blood cells. The nontoxin-producing strain of *Staphylococcus albus* was isolated in May, 1937, from a furuncle in the nose. No lysis of the red blood cell suspension occurred when 0.8 c.c. of the supernatant fluid from this culture was added.

The staphylococci were grown on agar slants for eighteen to twenty-four hours immediately preceding the animal inoculation. The washed bacteria were suspended in a physiologic salt solution. The suspensions of the organisms were brought to approximately the same concentration. Injections of 0.05 c.c. of these suspensions were made subcutaneously on each side of the abdomen of white mice. Twenty were given the suspension of the nontoxin-producing strain, and 26 the suspension of the toxin-producing strain. Two mice from each of three groups, namely, those receiving the toxin, the toxigenic and nontoxigenic strains of staphylococci were killed after thirty minutes, one, one and one-half, two, three, four, five, six, eight, and ten hours. Two mice inoculated with the toxin-producing strain were also killed after eleven and twelve hours.

Mice were passively immunized by two injections of 0.5 c.c. of staphylococcus antitoxin* given intraperitoneally at eighteen hours and at two hours prior to the subcutaneous inoculation of the washed bacteria. Groups of four of these immune mice were killed after intervals of three, six, and ten hours.

A 27 gauge needle was used for inoculating the bacteria. The abdominal wall in the area of the lesion was removed and placed in a 5.0 per cent solution of Zenker acetic acid immediately after the animals were sacrificed. The sections were prepared and stained with hematoxin and eosin. Select sections were stained according to Giemsa's method.

EXPERIMENTAL

Lesion Produced by Staphylococcus Toxin in Normal Mice.—The mice inoculated with the staphylococcus toxin appeared slightly less active than the controls. All of them survived the effect of the inoculum and were killed. The abdominal wall in the area of the inoculation was macroscopically edematous and hyperemic after three to four hours. The blood vessels were dilated on the peritoneal surface. Microscopically the vessels were dilated after thirty minutes. At this time the subcutaneous tissues were edematous and remained swollen during the remainder of the experiment. The muscle in the abdominal wall showed focal hyaline and nonstriated areas, and necrosis after ninety minutes. The injury to the tissue apparently did not progress after the first two hours. Polymorphonuclear leucocytes were the characteristic cells present. After thirty minutes these cells lined the lumina of the dilated vessels, and were present in the adjacent tissue. Only a few leuco-

*Supplied through the courtesy of Lederle Laboratories.

cytes were found in the edematous and necrotic tissue during the first three hours after inoculation. Many of these cells were pyknotic and lyzed. After six hours the leucocytes appeared more numerous and better preserved. At this time they seemed to be in greater number about the necrotic muscle tissue.

Lesions Produced by a Nontoxigenic Strain of Staphylococci in Normal Mice.—The mice appeared normal following the inoculation. The early gross lesions were similar to those which occurred with the toxin. The bacteria were more or less localized in the subcutaneous tissue. The muscle never showed any evidence of necrosis, and the subcutaneous tissue showed only a small amount of necrosis at the end of the experiment. Polymorphonuclear leucocytes were the characteristic cells in the inflammatory process. After thirty minutes these cells lined the lumina of the dilated vessels and infiltrated the adjacent tissue. As time progressed the leucocytes appeared to migrate toward the bacteria. After three hours the leucocytes had collected about the organisms and infiltrated the adjacent tissue. After eight hours the leucocytes had invaded the entire area previously occupied by the bacteria, and practically all the latter were phagocytosed. Little change occurred in the reaction during the last two hours of this experiment.

Lesions Produced by a Toxigenic Strain of Staphylococci in Normal Mice.—A majority of the 26 mice that received these organisms were weak and would not eat after three hours. Four of the group died five to ten hours after the injection. Pathologic studies were made only on the mice that were sacrificed.

Macroscopically the abdominal wall in the inoculated areas was edematous and hyperemic after three hours. As the experiment continued, the edema progressively spread to involve the greater portion of the wall. After six to eight hours the skin was pale yellow in color. Microscopically the changes after thirty minutes were similar in only a few respects to those in which the nontoxin strain of staphylococci was injected. Polymorphonuclear leucocytes were present in the tissues about the blood vessels in the areas where there were no bacteria. Only a few leucocytes apparently ever infiltrated the zone occupied by the organisms, and these were destroyed very rapidly. Phagocytosis was not a conspicuous process at any time during this experiment (Fig. 1*C*).

Although it was impossible to completely control the site of inoculation, there seems to be no doubt that the bacteria invaded very quickly the surrounding tissue. The leucocytes failed to localize the organisms after eight hours as they did the nontoxin-producing strain of staphylococci. Extensive necrosis occurred after five hours (Fig. 1*A*). Bacteria invaded the walls of some of the smaller blood vessels. Injured and degenerated leucocytes infiltrated the tissue in the abdominal wall. Staphylococci and leucocytes were present along the peritoneal surface.

Lesions Produced by a Nontoxigenic Strain of Staphylococci in the Immune Mice.—The mice were weak and had severe rigors within five minutes after receiving the staphylococcus antitoxin. After two hours they appeared nor-

mal. Three hours after the inoculation of the bacteria the abdominal wall was edematous and hyperemic. This reaction slowly progressed during the remainder of the experiment.

The histologic changes in these mice were very similar to those in the normal. Some of the bacteria appeared to form small clusters. It is difficult to determine whether or not there was greater agglutination of the bacteria

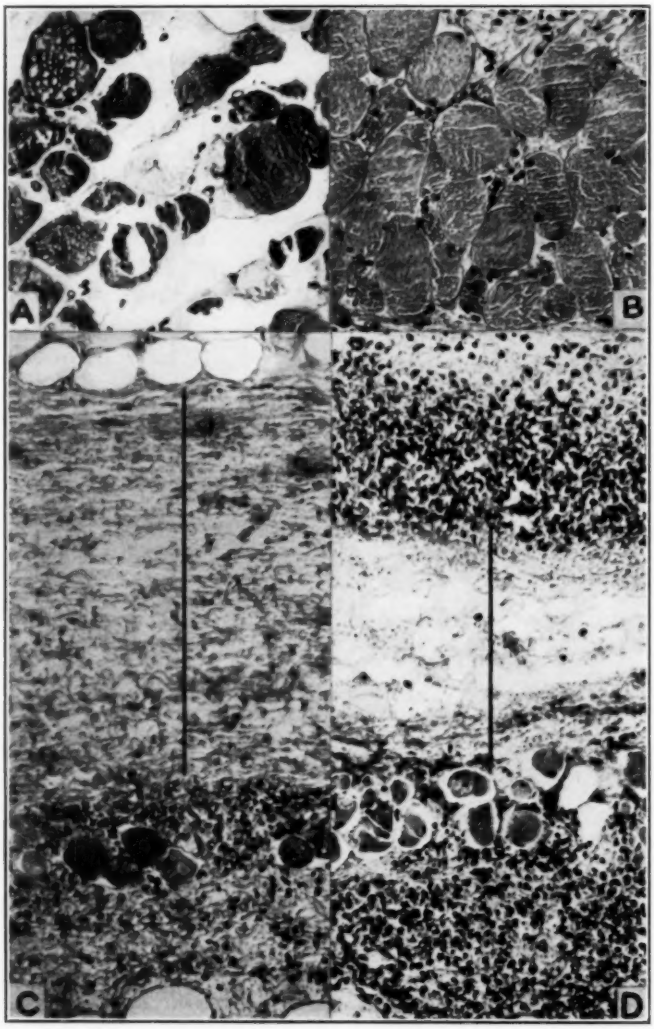

Fig. 1.—*A*, The abdominal muscle is severely injured by the toxin liberated by the staphylococcus in vivo. This normal mouse was killed five hours after injecting subcutaneously 0.05 c.c. of a toxigenic strain of staphylococci.

B, The abdominal muscle is normal in the passively immunized mouse six hours after receiving the same suspension of bacteria as shown in *A*.

C, Only a few polymorphonuclear leucocytes are present after five hours about the toxin-producing staphylococci in the subcutaneous tissue of this normal mouse. The bacteria are present in the greatest number in the subcutaneous tissue in the zone indicated by the line.

D, The bacteria are more localized and there is a marked increase of leucocytes in the subcutaneous tissues of this passively immune mouse when compared with the animal shown in *C*. A majority of the staphylococci are present in the subcutaneous tissue in the zone indicated by the line. Section removed after six hours. (Hematoxylin and eosin. ×275.)

in the immune than in the normal mice. There were many more polymorphonuclear leucocytes, and there was greater phagocytosis in the immune animals than in the normal mice.

Lesions Produced by a Toxigenic Strain of Staphylococci in Immune Mice.—The mice in this group looked very similar to those receiving the nontoxin-producing strain. Macroscopically the lesions in the two groups of immune animals were very much alike. Microscopically the toxin-producing strain of staphylococci never invaded the adjacent tissues as extensively as they did in the normal. Three hours after injecting the bacteria there were many polymorphonuclear leucocytes present. Apparently there was also less destruction of cocci and greater phagocytosis of the bacteria by the leucocytes in the immune animals than in the normal (Fig. 1*D*). These toxin-producing organisms remained localized in the immune mice the same as the nontoxin-producing strain. At no time was there extensive edema and necrosis like that which occurred in the normal mice (Fig. 1*B*). The only necrosis observed in the muscle was found in a few areas where there was an extraordinary large number of staphylococci.

DISCUSSION

The absence of any polymorphonuclear leucocytes in the tissues of normal mice for approximately three hours after the injection of staphylococcus toxin, and the presence of local necrosis during this time may indicate either that the leucocytes are not present in the tissue or that they are destroyed by the toxin. During the first three hours following the subcutaneous inoculation of toxin, apparently all of it either unites with the tissue or is removed by the blood or lymph. Leucocytes apparently are not destroyed by the toxin after this time as they enter the areas to phagocytose the cellular debris resulting from the action of the toxin on the tissue.

The toxigenic strain of staphylococcus produces lesions in the normal mice similar to those produced by staphylococcus toxin alone. In view of this it appears very likely that the staphylococcus liberates a substance in vivo which injures the tissues. The effects of this substance are not as evident when animals are passively immunized with staphylococcus antitoxin.

In the absence of staphylococcus antitoxin the toxin-producing strain invades the necrotic tissue. The toxin liberated in vivo produces necrosis of the tissues and destroys the leucocytes. In passively immune animals the bacteria apparently remain localized, and they are more quickly phagocytosed. The toxin liberated is evidently immediately neutralized by the antitoxin.

The reaction of the tissues to a nontoxin-producing strain of staphylococci is very similar in both the normal and immune mouse. There may be, however, more leucocytes and greater phagocytosis in the latter. It is difficult to determine from histologic studies whether or not there is an increase in the number of leucocytes since there is some variation in normal mice. There are many observations, however, which show that there is greater phagocytosis of bacteria in animals immunized with vaccines than there is in the normal.[9, 10]

The observations made in this study on the local tissue reactions with toxin and nontoxin-producing strains of staphylococci are similar to those of Tsuda using virulent and avirulent streptococci and pneumococci.[10]

There are a larger number of leucocytes and a greater phagocytosis in the immune than in the normal mice. Apparently the antitoxin neutralizes the toxin and thus prevents the destruction of the leucocytes. The increased phagocytability may be the result of a longer survival of the leucocytes permitting them to continue to phagocytose the bacteria.

Staphylococci are present in clusters in the mice given staphylococcus antitoxin. Similar groups of bacteria are also observed in some of the normal animals. This clumping of the bacteria may have resulted from inoculating groups of organisms directly into the tissue. Staphylococci, as is well known, have a tendency to grow in groups in vitro.

Ramon,[3] in discussing the effect of staphylococcus antitoxin, expressed the opinion that it protects the cells and tissues against the necrotizing action of the staphylococcus poison and thus makes a less favorable medium for the growth and multiplication of the organisms. The leucocytes, in the presence of antitoxin, are able to phagocytose the bacteria and debris. Staphylococcus antitoxin is, therefore, indirectly bactericidal. The results of this experiment confirm Ramon's opinion and indicate that greater phagocytosis of a toxigenic strain of staphylococci occurs in mice passively immunized with staphylococcus antitoxin than occurs in normal mice.

The mechanism of the immunity produced by staphylococcus antitoxin in local staphylococcus infections appears to be primarily the result of the neutralization of the toxin. The leucocytes apparently survive for a longer period in the immune animal and continue to phagocytose the bacteria. Staphylococci may also be more easily phagocytosed in the presence of immune sera. The importance of leucocytes in staphylococcus immunity has been shown by Cannon and Pacheco[2] in animals immunized with staphylococcus vaccines. The present study also shows the importance of leucocytes in animals passively immunized with staphylococcus antitoxin.

SUMMARY

Mice passively immunized with staphylococcus antitoxin and given staphylococci subcutaneously show more polymorphonuclear leucocytes and greater phagocytosis than the controls without antitoxin. If the staphylococcus produces a toxin in vivo it is neutralized by the antitoxin. The toxin-producing strains of staphylococci remain localized in the immunized mice similar to a nontoxin-producing strain in normal mice.

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GERMICIDAL PROPERTIES OF THE OXIDES OF NITROGEN*

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SO FAR as we have been able to determine, there has been no work published in literature that has detailed the systematic study of the germicidal action of the oxides of nitrogen.

Review of the literature does reveal that the oxides of nitrogen have been used with excellent results in the treatment of infected wounds, osteomyelitis, chronic empyema, and tuberculous sinuses. Much of this work has been done and published by Sweek,[1] of Phoenix, Ariz. He and others have been fully aware of the remarkable bactericidal properties of the oxides of nitrogen but have not taken up the work involving a purely bacteriologic study.

In 1903, J. N. Alsop, of Owensboro, Ky., experimented with a machine for aging flour. This machine consisted mainly of a high tension electric arc, setting up vibrations in the air. During his experiments he occasionally noticed dead flies around the machine. Brockett, of Princeton, discovered that the gas from a similar machine possessed definite bactericidal powers. Alsop cured dogs of mange by placing them in a chamber containing the electrified air.[2]

Dr. Sternon, of Owensboro, Ky., devised and used a machine in which a pine block was placed in the ionizing chamber and inserted into the electric arc at intervals. This type of machine was used also by the late J. B. Murphy and his associates at the Murphy Clinic. It was thought that the pine block would add certain organic matter to the gas which would enhance its bactericidal power.

Dr. Lowell, at the Mercy Hospital in Cincinnati, now uses a machine in which the gas comes in contact with a pine stick. He believes that the organic matter derived from the pine is an essential factor in preparation of the germicidal gas.

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*From the Lois Grunow Memorial Clinic, Phoenix.
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Dr. W. O. Sweek substituted pine oil for the pine block. Air was passed over the pine oil before entering the ionizing chamber. He, however, discarded the idea of the organic matter several years ago, as he has seen no difference in the bactericidal power nor any difference in the rapidity with which infected wounds heal. He now uses a machine in making the gas which does not bring organic matter in contact with the arc.

In our experiments we have used a duplicate of Dr. Sweek's machine, a description of which is included in this report.

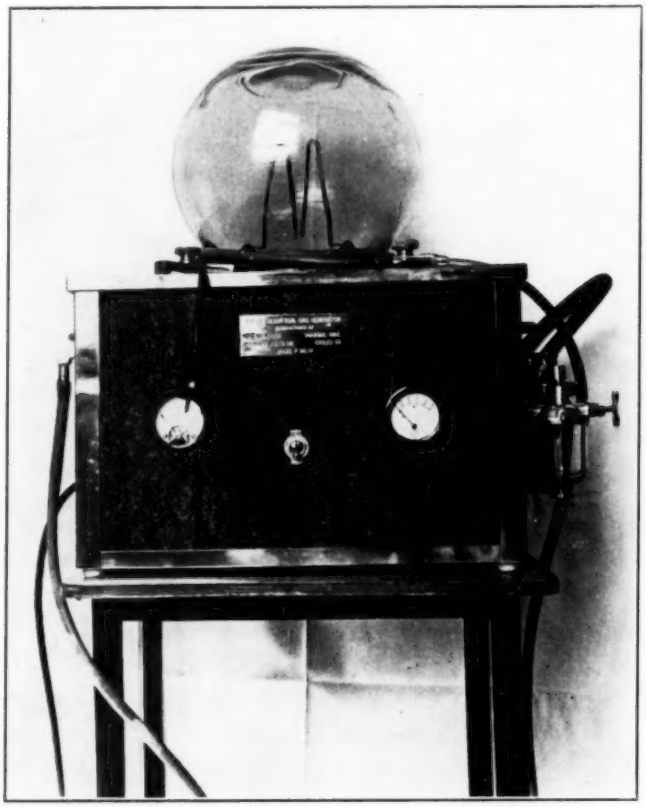

Fig. 1.—The gas machine used in the experiments. It will be noted from its description that it is very compact and easy to transport. While the machine is in operation, it is no more expensive to operate than an ordinary electric iron.

In taking up the study of any antiseptic or germicide for general use, one must consider several factors; namely:

1. The availability and economical status of the antiseptic.
2. What harm can be done to a patient or tissues with which it comes in contact during its administration?
3. Does it possess definite antiseptic and bactericidal qualities?

In answer to the first factor we can safely say that it is easily and economically produced. The second factor has been discussed in previous publications.[1] However, we may state here that this gaseous antiseptic correctly

used within its limitations is harmless. The discussion and study of the third factor will be the purpose of this paper.

For a more thorough understanding of the antiseptic under consideration, we give a brief description of the preparation of the gaseous antiseptic used in these experiments.

When compressed air is passed through an electric arc of sufficient amperage and voltage, the oxygen and nitrogen of the air undergo a certain chemical combination. The exact sequence and the end products of these changes are not definitely known. However, we do know that the oxides of nitrogen are produced and that the resultant gas, in the presence of moisture, forms nitric acid. We know also that when an equilibrium is reached, the percentages of the different oxides of nitrogen vary with several conditions; namely: (1) the amperage and voltage used; (2) the width of the spark; (3) the pressure of the compressed air as it enters the ionizing chamber; (4) the distance from the arc at which the gas is taken; and (5) the temperature of the gas. Variations in these factors have a very definite influence on the germicidal qualities of the gas as well as its effect on the tissue cell.

We believe that the machine we have used in our experiments has the above factors so adjusted as to have the maximum power as a germicide with a minimum effect on the tissue cell.

Fig. 1 shows the machine used in evolving the germicidal gas used in our experiments. The dimensions of the cabinet are 16½ by 10½ by 10 inches. Air enters through the right end panel. The gas outlet is through the left end panel and the electrical attachment cord. The top panel carries a 10 liter ionizing chamber, air nozzle, and electrodes. Air at 2 to 3 pounds pressure enters through the right end of cabinet and proceeds on into the ionizing chamber through ⅛ inch air nozzle directly below electrodes. The amperage is 60 Ma. at 12,000 volts. As the air passes through the arc, the oxygen and nitrogen undergo certain chemical combinations. It makes its escape from the ionizing chamber through an outlet at the left end of the cabinet. At the end of a 5/16 inch 10 ft. rubber hose, a glass nozzle or catheter is attached for treatment purposes.

The clinical germicidal and fungicidal effect of the gas having been demonstrated in many cases, it was, of course, obvious to us that these qualities could be demonstrated in the laboratory by bacteriologic experimental methods. We have repeatedly demonstrated this to be true during the past several years by numerous and varied cultural experiments with many different kinds of bacteria and fungi. In every laboratory experiment the high germicidal and fungicidal effect of the gas was demonstrated even more convincingly than in the clinical cases treated.

Tables I to V show a series of carefully controlled bacteriologic experiments. These experiments were carried out with pure cultures of various bacteria obtained from Parke, Davis and Co. It was thought best to employ strains of bacteria obtained from outside sources in order to avoid the possibility of encountering weakened or attenuated strains accidentally isolated by us or resulting from numerous necessary subcultures while isolating pure strains in our own laboratory.

TABLE I

Inoculations were made from saline emulsion and forty-eight hours' agar growths. They were gassed and allowed to incubate twenty-four and forty-eight hours before the readings were taken. It will be noted that the *Streptococcus pyogenes*, which is ordinarily killed quickly, proved to be a good grower, as recommended by the pharmaceutical house.

BACTERIA BRAIN VEAL AGAR	15 SEC.	30 SEC.	45 SEC.	60 SEC.	75 SEC.	90 SEC.	105 SEC.	120 SEC.	135 SEC.	150 SEC.	CONTROL
Staphylococcus aureus	+	+	+	No growth	--	--	--	--	--	--	++++*
Bacillus coli	7 colonies	5 colonies	2 colonies	--	--	--	--	--	--	--	++++
Bacillus pyocyaneus	3 colonies	2 colonies	2 colonies	--	--	--	--	--	--	--	++++
Streptococcus pyogenes	++++	+++	+++	++	++	+	+	--	--	--	++++

*Solid growth.

TABLE II

The strain of *Streptococcus pyogenes* is very resistant and blood agar cultures are more resistant to the gas on account of the lack of moisture. It will be noted also that the same strain was completely killed in one and one-half minutes in a saline suspension, as shown in Table III.

	5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	CONTROL
Streptococcus pyogenes 48-hour culture brain veal agar	++++	+++	++	--	--	++++

TABLE III

The bacteria are killed somewhat more quickly in the presence of more moisture.

	30 SEC.	1 MIN.	1½ MIN.	2 MIN.	3 MIN.	4 MIN.	5 MIN.	CONTROL
Streptococcus pyogenes suspension in normal saline	++	+	2 colonies	--	--	--	--	++++

TABLE IV

Petri dishes were gassed and left open for twelve hours exposed to the air, then recapped, and incubated for twenty-four to forty-eight hours. It will be noted that the gas caused some change in the media that prevented growth of mold and bacteria. The Petri dishes were gassed five, ten, fifteen, twenty, and twenty-five minutes, respectively, before exposure to the air.

	5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	CONTROL
Petri dishes nutrient agar	--	--	--	--	--	++++

TABLE V

About five minutes of gassing of the Petri dish is necessary before the surface of the media becomes bactericidal.

	1 MIN.	2 MIN.	3 MIN.	4 MIN.	5 MIN.	CONTROL
Petri dishes nutrient agar	3 mold and bacteria	7 colonies bacteria	5 mold	4 mold	2 mold	Colonies of mold and bacteria

The *Streptococcus pyogenes* strain was obtained after the first strain furnished by them had proved to be lacking in growing qualities on subculture. The one used in this experiment was recommended by them as being a good "grower." We found that their prediction was accurate, and it will be noted that this culture was more resistant to the gas than any of the other strains of bacteria. It will also be noted, however, that all the bacteria were quickly killed by the gas. The media used in these experiments were the ordinary Difco products, the brain veal agar having a pH of 7.65+ while the nutrient agar had a pH of 6.7+. It was interesting to note that even with prolonged treatment of the media by the gas the reaction of the media was not altered to the acid side sufficiently enough to be shown by any of the ordinary indicators. It is believed that this fact may somewhat explain the nonirritating quality of the gas when used over prolonged periods in the treatment of infected wounds and sinuses.

The Petri dishes used in this test were of standard size, and the agar slants were contained in test tubes 5/8 inch by 6 inches. The gas was applied to the surface of the slanted media through a rubber or glass tube with a tip small enough to reach entirely into the apex of the slant. If this was not done carefully, it was found that a pocket of air would be produced in this recess, preventing the gas from reaching the bacteria here. It was also found important to move the tip about slightly while applying the gas in order that no bacteria would be imprisoned underneath the tip, thus preventing access to them by the gas.

It has been suggested that the gas passing over the surface of the media might exert a drying effect, besides blowing away some of the bacteria. With this possibility in mind, a series of experiments were conducted. Air alone was passed over the surface of the media in the same manner and under the same conditions as obtained when the gas was used. There was a luxuriant growth on each tube, showing that the drying or blowing effect was not a factor in these experiments.

One is impressed throughout these experiments with the high bactericidal power of this gaseous antiseptic. There has been much speculation, and

many theories have been advanced as to what particular factor or group of factors is responsible for the germicidal power of the gas.

Dr. Lowell's theory is that the beneficial effects are due to electronic changes brought about in the tissues, resulting in a more normal state of electrical balance, thus enabling the tissues to fight infection more effectively. This explanation applies to wounds that are being treated with the gas and is not an explanation of its germicidal activity in bacterial cultures.

Dr. Sweek is of the opinion that its germicidal activity is due to the formation and deposition of nitric acid and other compounds as yet unanalyzed, the bacteria being destroyed by dissolution or injured to such an extent that death of the bacteria results.

It has been noted that the amount of nitric acid deposited on the media is very small as it does not change the pH enough to be shown by the ordinary indicators. We do know, however, that if this gas is passed through distilled water for one to two hours, the pH is changed considerably toward the acid side, as shown by titrable acidity. We also know that the gas is not effective in the absence of moisture, but is this not true of any gaseous, liquid, or semisolid germicide? From our clinical experience we may add that after gassing an open wound for one hour there has not been enough nitric acid deposited to irritate or injure tissue cells. On the contrary, the wound heals rapidly and cultures from the wound are sterile after two or three such treatments. It has been stated by Dr. Leo Loeb, and corroborated by others, that small amounts of dilute acid enhance the action of the phagocyte and stimulate granulation tissue formation. Further, in the treatment of infected wounds with this gas, the small amount of acid formed helps to dissolve fibrin and other necrotic material that might retard wound healing.

It is possible that some nascent oxygen is formed in the gas which exerts its benefit as an oxidizing agent. We feel that small amounts of hyponitrous acid are formed which further enhance the oxidizing power of the gas.

CONCLUSIONS

1. Compressed air passed through an electric arc, as described in this report, produces a mixture of gases which is a very effective bacteriostatic and bactericidal agent.

2. The bacteriostatic and bactericidal action of this gas is effective in fifteen seconds' exposure.

3. This gas is also fungistatic and fungicidal.

4. The vegetative forms of bacteria and fungi are the least resistant to the gas, the spore forms being very resistant.

5. We believe that the bactericidal power of this gas is due to formation and deposition of minute amounts of nitric acid and other agents contained in the gas that as yet have not been analyzed.

6. The gas is easily and economically produced and should find its greatest field of usefulness in the treatment of infected wounds and chronic draining sinuses, particularly osteomyelitis and tuberculous sinuses.

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ULTRAVIOLET IRRADIATION AND VITAMIN C METABOLISM*

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WITH the advance of knowledge, interest in vitamin C has shifted from consideration of its importance as an antiscorbutic agent to a fuller appreciation of its function in the physiologic defense mechanism against various infections and intoxications. It is now common experience that most infectious diseases are accompanied by a loss of the vitamin from the body; moreover, it is often possible, experimentally and clinically, to enhance resistance or to hasten recovery by raising the vitamin levels through the administration of ascorbic acid.[1]

The significance of low-grade C deficiencies, or hypovitaminoses, in the epidemiology of infectious diseases is far from being understood; indeed, the very complexity of the problem presents great difficulties for systematic exploration. However, it remains an inviting task to attempt to correlate seasonal variations in susceptibility, as indicated by the periodic oscillations of mortality and morbidity, with cyclic changes of vitamin C metabolism. Unlike the mode of utilization of other vitamins that are stored in considerable reserves in the body, the vitamin C intake and outflow represents a delicately attuned system which undergoes continuous alteration and adjustment. The changes in this system may be measured directly by the degree of C saturation of blood and tissues, on the one hand, and by the rate of C elimination through peripheral secretions and excretions (milk, sweat, tears, saliva, and urine) on the other. That the maintenance of this balance is subject to seasonal influence is suggested by numerous observations which need hardly be related here in detail.[2] It is as yet uncertain to what extent these phenomena are brought about by exogenous or by endogenous factors, individually or collectively; but there are good reasons for assuming that, apart from differences in dietary intake, environmental conditions connected with the operation of climatic constants must play an important part.

Exposure of guinea pigs to high temperatures, for instance, is followed by a diminution of vitamin C in the liver, brain, and adrenals, and the ad-

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ministration of ascorbic acid is said to protect the animals against the lethal effect of heat damage.[3] Similar were the findings of Zook and Sharpless,[4] who not only observed a rapid depletion of C stores in animals with elevated temperatures but also showed that artificial fever reduces the urinary excretion of the vitamin in man. Exposure of guinea pigs to ultraviolet light caused either an increase or a decrease of the ascorbic acid content of the skin, depending upon whether irradiation was brief or prolonged (Castellini[5]); similarly, Wels's[6] experiments demonstrated an actual increase of the sulfhydryl groups in swine skin following ultraviolet raying. These results are in good agreement with the observation of Holtz and Woellpert[7] that the clinical symptoms of scurvy were milder in C deficient guinea pigs, which had received small doses of ultraviolet light, than in nonirradiated controls, even though both sets of animals presented the fully developed picture of scorbutic lesions. The effect of ultraviolet light on vitamin C metabolism in man has been investigated by several authors, but the results are not entirely in accord. While Schade[8] reported that vitamin C administration inhibited both the erythema and pigmentation resulting from ultraviolet irradiation, Urbach and Kral[9] obtained a definite light-protective effect only by combining vitamin C doses with local application of bergamot oil. Unpublished data by Dr. Irving S. Wright,[10] to which we may refer by permission of the author, indicate that the production of an extensive ultraviolet light erythema was followed by an abrupt retention of urinary vitamin C in one out of three subjects; this individual was red-haired, fair-skinned, and could tolerate much less ultraviolet light than the other two subjects. Drigalski,[11] on the other hand, reported no change in the rate of urinary excretion of vitamin C as the result of ultraviolet irradiation. Since ultraviolet light in excessive doses provokes a severe tissue burn, it is not surprising to note that both Stolfi[12] and Einhauser[13] observed a precipitous drop in the vitamin C levels of various organs—especially marked in the adrenal—in rabbits or in guinea pigs whose extremities had been scalded by immersion in hot water. Einhauser also described the lifesaving effect of cortin and vitamin C, either together or alone, when given to animals suffering from experimental burns. The above quotations suffice to delineate the scope of the work that has been done in this field and, at the same time, serve to emphasize the limitations of our present knowledge.

We have been particularly interested in the relationship that seems to exist between vitamin C metabolism and the effect of ultraviolet light in the production of disease, when irradiation transcends the threshold of physiologic stimulation. Clinical experience has amply demonstrated the potential danger of excessive doses of ultraviolet light in the production of critical changes in capillary fragility which may be instrumental in causing hemorrhage in vitamin C deficient patients suffering from pulmonary tuberculosis or gastric ulcers. But apart from such accidents, light damage may possibly play a more fundamental part in the pathogenesis of certain infectious diseases that reach an unexplained seasonal peak of epidemicity during

the summer months. Thus, the coincidence of sunburn following unrestrained exposure at beaches and swimming pools and the occurrence of cases of poliomyelitis are so frequent and almost pathognomonic that some epidemiologists have seized upon this sequence as indicating the water-borne nature of poliomyelitic infection; in doing so, the obvious fact was overlooked that the individuals bathed not only in the water but also in the sun. The suspected correlation becomes even more compelling when one contrasts the rarity of the disease in southern climes, where the even presence of radiation throughout the year provides for a protective tanning, with its massed incidence in northern latitudes in which the full force of summer sunlight must be borne by an unprepared skin. Conceivably, the apparent racial difference in natural resistance to poliomyelitis that separates the highly susceptible white races from the relatively less susceptible colored races (black and yellow), may in some measure be related to the abundance of pigment in the skin of the latter, acting as a protection against the hypothetical photodynamic injury.

EXPERIMENTAL

It was the object of this study to determine whether or not exposure of laboratory animals to ultraviolet irradiation was followed by a significant change in the vitamin C content of representative tissues. The animals employed in this work were albino guinea pigs, rabbits, and rhesus monkeys; the tissues examined were brain, liver, spleen, and adrenal gland. As the source of ultraviolet light we used a mercury vapor lamp, energized by high frequency currents (Lepel) and emitting a spectrum in which the longer ultraviolet waves, in the range from 2,800 to 3,100 angstrom units, predominate. The same lamp had been used in previous studies on the inactivation of poliomyelitis virus by ultraviolet light.[14] Animals, whose backs had been cleanly shaven, were placed under the lamp at a distance of about 30 cm. from the burner and exposed to the light for from thirty to sixty minutes. After intervals of one and eighteen hours, respectively, the animals were killed, and their organs examined for vitamin C content. For purpose of control, unrayed animals, which had previously been kept on the same dietary regime, were sacrificed simultaneously, and vitamin C titrations of their organs were carried out, side by side, with those of the organs of rayed animals. Preliminary diets were planned to cover the approximate range from inadequacy to adequacy. Duplicate animals were used in each test, pooling the organs of pairs of guinea pigs for vitamin C analysis. The amounts of reduced vitamin C in the tissues were determined by titration with indophenol (sodium 2, 6-dichloro-benzenoneindophenol, Eastman Kodak Co.). Because of its simplicity and accuracy we adopted the modified method of Bessey and King,[15] the experimental error of which is estimated between 5 and 10 per cent; technical details may be found in an earlier paper in which the same method was employed satisfactorily for the determination of vitamin C levels of monkey tissues.[16] The results of the various titrations are brought together in Table I which also gives additional data relating to the experimental setup.

TABLE I

EFFECT OF ULTRAVIOLET IRRADIATION ON VITAMIN C LEVELS OF TISSUES

EXPERIMENT*	ANIMAL	DIET	DURATION OF IRRADIATION	INTERVAL AFTER RAYING	VITAMIN C CONTENT OF ORGANS (MILLIGRAM PER GRAM)			
					BRAIN	LIVER	SPLEEN	ADRENAL GLAND
1	Guinea pigs	Inadequate	Control	--	0.18	0.07	0.25	0.62
	Guinea pigs	Inadequate	Control	--	0.16	0.08	0.16	0.39
	Guinea pigs	Inadequate	Control	--	0.13	0.08	0.16	0.36
	Guinea pigs	Inadequate	Control	--	0.14	0.08	0.23	0.57
	Guinea pigs	Inadequate	30 min.	1 hour	0.16	0.09	0.22	0.92
	Guinea pigs	Inadequate	30 min.	18 hours	0.15	0.07	0.15	0.36
	Guinea pigs	Inadequate	60 min.	1 hour	0.13	0.09	0.21	0.46
	Guinea pigs	Inadequate	60 min.	18 hours	0.14	0.09	0.24	0.40
2	Guinea pigs	Adequate	Control	--	0.20	0.25	0.54	1.44
	Guinea pigs	Adequate	Control	--	0.17	0.19	0.34	1.02
	Guinea pigs	Adequate	Control	--	0.19	0.26	0.50	1.47
	Guinea pigs	Adequate	Control	--	0.17	0.16	0.41	0.96
	Guinea pigs	Adequate	30 min.	1 hour	0.20	0.25	0.50	1.35
	Guinea pigs	Adequate	30 min.	18 hours	0.16	0.13	0.32	1.09
	Guinea pigs	Adequate	60 min.	1 hour	0.19	0.28	0.53	1.42
	Guinea pigs	Adequate	60 min.	18 hours	0.17	0.16	0.37	0.98
3	Rabbits	Adequate	Control	--	0.19	0.12	0.52	1.76
	Rabbits	Adequate	Control	--	0.20	0.16	0.41	2.29
	Rabbits	Adequate	30 min.	1 hour	0.19	0.13	0.49	1.92
	Rabbits	Adequate	30 min.	18 hours	0.21	0.18	0.53	1.93
	Rabbits	Adequate	60 min.	1 hour	0.19	0.16	0.52	1.77
	Rabbits	Adequate	60 min.	18 hours	0.21	0.18	0.49	2.07
4	Monkeys	C-stored	Control	--	0.16	Not done	0.27	0.51
	Monkeys	C-stored	30 min.	1 hour	0.15	Not done	0.36	0.64

*All experiments were done in June, 1938.

It will be seen from Table I that exposure of guinea pigs, rabbits, and monkeys to ultraviolet light for from thirty to sixty minutes caused no change whatsoever in the vitamin C content of the brain, liver, spleen, and adrenal gland. The average figures for the rayed and unrayed groups, respectively, were almost exactly identical, the range relatively small. This was true for guinea pigs which entered the experiment with partially depleted vitamin C concentrations as well as for guinea pigs which had been permitted to accumulate reserves by previous high level feeding.

The outcome of the above experiments was so clearly negative, irrespective of whether one was dealing with animals capable or incapable of synthesizing vitamin C, that there could be no doubt about the inefficacy of our method of irradiation in disturbing vitamin C metabolism. It was quite evident, however, that our rayed animals at no time exhibited any erythema on the skin despite the fact that the same lamp, even when operated at a greater distance, induced a very intense erythema on human skin after only three minutes' exposure. Marked irregularities in attempts to produce an erythema on the skin of guinea pigs by powerful ultraviolet irradiation have previously been reported by Ellinger.[17] The reason for this discrepancy between the reactivity of human and animal skin is not clear, and we have no explanation to offer for it. However, it occurred to us that the failure of the animals to develop a burn from the ultraviolet light might possibly account for the absence of vitamin C changes; conversely, if a burn were produced

by another more drastic method, such changes might ensue. We have, therefore, taken another group of guinea pigs, kept one-half for controls, and cauterized the shaven skin of the other half by the brief application of a searing knife over an area of approximately 2 cm. by 2 cm. The lesion thus provoked was entirely superficial, and, while not leading to any severe tissue destruction, proved sufficiently intense to cause discolorization of the epidermis with very slight scab formation. This experiment was carried out three times, once in summer and again in the winter and spring, in each case using duplicate animals, all of which had been kept before on an adequate diet. The results of the three vitamin C assays appear in Table II.

TABLE II

EFFECT OF SKIN CAUTERIZATION ON VITAMIN C LEVELS OF TISSUES

ANIMAL	DIET	CAUTERIZATION	INTERVAL AFTER CAUTERIZATION	VITAMIN C CONTENT OF ORGANS (MILLIGRAM PER GRAM)			
				BRAIN	LIVER	SPLEEN	ADRENAL GLAND
Guinea pigs	Adequate	Control*	--------	0.15	0.16	0.38	1.04
Guinea pigs	Adequate	Cauterized	18 hours	0.13	0.07	0.15	0.38
Guinea pigs	Adequate	Control†	--------	0.11	0.12	0.25	0.51
Guinea pigs	Adequate	Cauterized	18 hours	0.09	0.06	0.18	0.35
Guinea pigs	Adequate	Control‡	--------	0.10	0.04	0.11	0.25
Guinea pigs	Adequate	Cauterized	18 hours	0.09	0.02	0.10	0.37

*Experiment done in June, 1938.
†Experiment done in February, 1939.
‡Experiment done in April, 1939.

It is clear from the data given in Table II that the burned guinea pigs experienced in all organs save one a more or less pronounced drop in vitamin C levels; this drop reached its greatest magnitude in the liver. The change was well marked in all three experiments, although the influence of seasonal variation made itself felt in a tendency for the vitamin C values of all tissues to run noticeably higher in the summer than in the winter and spring. A similar phenomenon was observed before in our vitamin C assay of monkey tissues.[16] The levels found for normal control tissues compare favorably with the range reported in the literature for guinea pigs that have been kept on an adequate diet.[18]

DISCUSSION

The experimental data presented in this paper demonstrate that prolonged ultraviolet irradiation of albino guinea pigs, rabbits, and monkeys, fails to bring about any change in the tissue levels of vitamin C as it also proves singularly ineffective in inducing an erythema on the shaven skin of the rayed animals. However, when a superficial burn is produced on the skin of guinea pigs by the local application of heat, a more or less marked diminution of the vitamin C stores occurs in all the organs tested. The latter observation is in complete harmony with similar findings of Einhauser.[13] It is possible that toxic, histamine-like substances, which have an adverse effect on adrenal function, originate from the burned tissue and require large amounts of vitamin C for their detoxification,[19] a process which may be analogous to the well-known

depletion of vitamin C following the necrotizing action of diphtheria toxin. The fact that ultraviolet irradiation, without inducing visible tissue damage in laboratory animals, also leaves the vitamin C stores intact is of particular interest in view of Wright's[10] observation that evidence of disturbed vitamin C metabolism occurred only in one individual who showed definite intolerance to ultraviolet light exposure. Evidently, a mechanism operates in some individuals which protects tissues against damage and preserves the vitamin C reserves in the tissues; vice versa, the absence of such a protective mechanism in other individuals, or its breakdown by overdosage of light, may initiate a derangement of vitamin C metabolism. This, in turn, may assume significance as a contributing factor in the lowering of natural resistance against certain infectious diseases.

SUMMARY AND CONCLUSIONS

1. Exposure of guinea pigs, rabbits, and rhesus monkeys to prolonged ultraviolet irradiation caused no change in the tissue levels of vitamin C.

2. Such irradiation failed to produce any evidence of erythema on the skin of the rayed animals.

3. The production of a superficial burn on the skin of guinea pigs by the local application of heat was followed by a more or less marked drop in the content of vitamin C of the brain, liver, spleen, and adrenal gland.

4. Excessive doses of radiant energy apparently cause a derangement of vitamin C metabolism only to the extent that they are capable of producing injury to the tissues.

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GONORRHEAL MYELITIS WITH ASSOCIATED PORPHYRINURIA FOLLOWING SULFANILAMIDE*

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MYELITIS is one of the rarer complications of gonorrheal infection. Grinker[1] states, "Gonorrhea has been known in several instances to cause a myelitis. Apparently the organism ascends the urinary tract and either enters the nervous system by way of the venous channels or directly along the nerves. It produces an actual inflammatory destruction of the lower end of the spinal cord and gradually ascends." Blumer[2] says, "In patients with paraparesis following gonorrhea there is often great difficulty in deciding whether the lesion is a neuritis or myelitis. There is little doubt that true myelitis may occur." Gowers[3] gave a vivid clinical description of a rapidly progressive case of gonorrheal myelitis with fatal termination. Phifer and Forster[4] collected 29 cases from the literature to which they added 2 of their own. Gray[5] reported 5 cases of myelitis and encephalomyelitis with autopsy report in one. He stated that probably 70 to 80 cases of myelitis, meningomyelitis, or encephalomyelitis have been reported in which the development was directly associated with gonorrhea. He found 64 cases in the literature. All but 3 were in males. In his own series 4 of the 5 patients were females. The autopsy report was on a female patient. The important findings were encephalomalacia limited to the fiber tracts of the internal capsule and the cerebellum, a few perivascular round cells, and unusual calcification of many of the smaller arterioles in the brain. He discussed the possibilities of direct infection, of associated virus infection or toxic manifestation. He thought the pathologic findings indicated a toxic effect being the most likely in the case studied by him. Search of available literature has disclosed very few additional reports. Marinesco, Dragenesco, and Chiser[6] discussed the development of a myelitic gonococcus vaccine reaction which is of some interest in connection with the case here reported.

The patient described below showed, along with the neurologic findings of myelitis, the presence of porphyrin pigments in the urine. Consequently, various laboratory studies were carried out along with clinical observation of the changes in the nervous system.

CASE REPORT

A white male shoe worker, aged 26 years, was admitted to Our Saviour's Hospital, 11:30 P.M. on Nov. 5, 1937. His present illness began October 20 with urethral discharge, redness at the urinary meatus, urinary burning, and frequency. He consulted a physician two days later when the diagnosis of urethritis was made. He returned to his physician on October 25 and received a hypodermic injection. A report from the physician indicated

*From the Norbury Sanatorium, Jacksonville.
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gonococcus filtrate was given on October 25 and October 28. The patient stated he started sulfanilamide on the earlier date, taking four tablets four times a day for three or four days. These made him sick and dizzy so he cut the dose to one tablet four times a day for three days, then increased it to two four times a day until the day before entrance. This would give a total dosage of 460 to 540 grains of sulfanilamide over ten or eleven days, depending upon whether the initial dosage was kept up for three or four days, a point on which the patient was not certain. He felt chilly, dull, and draggy on October 31 and went to bed the following day. The urine was observed to be reddish in color after the injections with nocturia noticed somewhere in that period. This patient first noticed numbness of his feet on November 3, and became paralyzed the next day. He had increasing difficulty in urinating and required catheterization on the morning of November 4 and again on the day of entrance. Constipation had been increasing, but when castor oil was given the day before entrance, control of the bowels was lost and was not regained. On November 2 the patient had a chill and fever; on two days following he had two chills; on day of admission, none. He had vertigo since November 2. His vision was not clear on November 4 but returned the following day.

The past history of the patient was not remarkable. He was an inside worker and worked at his present occupation as a cutter of lining leathers in a shoe factory for eight years. He did not come in contact with dyes; the leather was already treated when he received it. His family history was negative.

His admission temperature was 102°, pulse rate 120, respiration 24. He was of slight build, rather undernourished and toxic appearing, conscious yet dull. He was pale with a suggestion of cyanosis. Pupils reacted promptly to light and accommodation. The fundi appeared normal. There was a toxic odor to his breath. No abnormal findings were noted in examination of his heart or lungs. Blood pressure was 100/60. There was tenderness in the suprapubic region where a distended bladder was found. There was a slight amount of thick yellowish urethral discharge with some redness at the meatus. Rectal examination was negative save for a relaxed sphincter. Extremities showed slight capillary cyanosis of the nails and edema of the ankles.

Neurologic examination: Complete anesthesia existed below the twelfth dorsal segment with a burning paresthesia just above it. There was some complaint of paresthesia in the hands. Muscle joint and vibration sensation were absent in the lower extremities which exhibited a flaccid paralysis from the waist down. The reflex findings were absent jaw jerk, upper deep equal but diminished, lower deep absent, epigastric, abdominal, cremasteric and plantars absent, Babinski present bilaterally with a definitely suggested bilateral Gordon and Oppenheim. There was no neck sign or Kernig.

Laboratory studies: Urine. On November 6 a catheterized specimen of deep red, clear, acid urine, with a specific gravity of 1.024 was obtained; it contained a trace of albumen but no sugar; microscopic study revealed a few pus cells. All urine was saved for various special examinations noted below. A slight trace of sugar was found on November 11 and from November 16 to 29; at all other times tests for sugar were negative. Albumen varied from negative to trace throughout the period of hospitalization. Granular casts were seen on November 11, November 15 to 18, and on November 28. Pus cells varied from negative to two plus at different times. Tests for blood, bile, hemoglobin, diazo reaction, and iodine, salicylic acid, phenacetin, rhubarb, senna, phenolphthalein, and pyramidon on the pigmented urine on November 6 were all negative. Sulfanilamide determinations, using the method of Marshall, Emerson, and Cutting,[7] gave the following results:

DATE	TIME	URINE VOLUME	SULFANILAMIDE (TOTAL)
November 6	A.M.	720 c.c.	290 mg.
November 6	P.M.	480 c.c.	204 mg.
November 7	A.M.	960 c.c.	166 mg.
November 7	P.M.	960 c.c.	109 mg.
November 8	A.M.	960 c.c.	Too low to read

Spectroscopic examination of the urine tested by the simpler methods, as outlined by Mason, Courville, and Ziskind[8] and by Schumm[9] gave the following results:

DATE OF TEST	DATE OF URINE	METHOD	RESULT
November 8	November 6, A.M.	Garrod	Negative
November 8	November 7, A.M. and P.M.	Langnecker	Spectrum of acid hematoporphyrin Deep red fluorescence with ultraviolet light
November 9	November 6, P.M.	Garrod	Trace acid hematoporphyrin
November 9	November 7, P.M.	H. Fischer	Negative

The spectroscope used was a Spencer, students' type, the property of the Physics Department of Illinois College, with 60 watt frosted Mazda bulb at 24 inches and solution depth of 1 cm.

Blood. Blood counts made on November 6 showed hemoglobin 74 per cent (Dare), W. B. C. 7,800; R. B. C. 4,400,000, differential neutrophiles 57, small lymphocytes 40, large lymphocytes 2, and basophiles 1. Daily counts were made until November 29, then weekly counts until patient was discharged. Red cell count dropped to 3,600,000 by November 11 but gradually mounted to 5,000,000 the day before discharge. The Kahn test on blood serum was negative.

On November 7 the blood sulfanilamide was too low to read. On November 8 the sugar was 67 mg., urea 17.7 mg., creatinine 1.5 mg., uric acid 4.6 mg. per cent, and icterus index 100 plus.

Spectroscopic examination on November 8 indicated the presence of methemoglobin in diluted serum; on November 9 it was present in diluted whole blood.

Urethral smear on November 8 was positive for gonococci.

Spinal fluid:

DATE	CELL COUNT	GLOBULIN	WASSERMANN	LANGE
November 6	316	2 plus	Negative	Negative
November 8	32	1 plus	Negative	Negative
December 4	5	Negative	Negative	Negative

Cells in the fluid of November 6 were mostly lymphocytes. No organisms were seen.

Progress: The patient showed some intermittent elevation of temperature until ten days before discharge. Neurologic changes gradually cleared. Superficial sensation was barely noted over the thighs on November 11, but absent elsewhere. Differentiation of heat and cold, pain and touch was possible by November 15. Awareness of light touch as a burning paresthesia was present by November 18 along with a return of muscle joint and vibration sensation. Clumsy motion of the right leg and slight movement of the left toes were also noted that day. Three days later the patient was able to void a small amount of urine. Redness of the skin over the sacrum to the left of the midline was noted that day. This broke down to a trophic ulcer by November 25 but was healing well by December 3. On this date the knee jerks were present but the ankle jerks were still absent. Sensation was good. Muscle power was fair, muscular atrophy pronounced. Examination on December 16 showed superficial and deep sensation normal, all reflexes but the cremasterics present, sphincter control good. The patient was lifted into a wheel chair that day, and with assistance sat up for Christmas dinner. He left the hospital January 4, 1938.

Subsequent course. Strength gradually returned as nutrition improved. He returned to his occupation June 1 and worked regularly ever since. Recent neurologic examination showed no abnormal findings.

DISCUSSION

Several factors entered into the consideration of etiology of the myelitis in this case. Toxic effects of sulfanilamide on the nervous system had been reported. Porphyrinuria is in many instances associated with lesions of

the central nervous system. Photosensitization and dermatitis following sulfanilamide led to association between sulfanilamide, porphyrinuria, and the cord lesion as a possibility. Evaluation of all factors, as well as the progress, brought about the conclusion that the condition was a gonorrheal myelitis with associated porphyrinuria following sulfanilamide.

Most of the effects of sulfanilamide on the central nervous system have been seen in experimental animals receiving doses proportionately much larger than those ever given clinically. Excitement, muscular weakness, ataxia, and signs of stimulation and depression of the central nervous system with spastic rigidity and hyperesthesia of twelve to twenty-four hours' duration were seen in dogs studied by Marshall, Emerson, and Cutting. Longcope[10] states, "Many patients experience malaise, anorexia, dizziness, somnolence, nausea, headache and are depressed. Vomiting is not uncommon. These are symptoms which have been observed in animals intoxicated by the drug and are dependent, no doubt, upon a direct action of the chemical." Such symptoms and signs are different from the ones in the patient studied here. Optic neuritis was seen by Bucy[11] in a patient who developed central scotoma for red and blue, and relative scotoma for white, following one tablet of sulfanilamide, although it had been given previously. As noted in the findings reported here, the patient said that vision was blurred the day before admission but that it had cleared up. Rather careful examination of the fundi showed no changes, but no perimetric readings were made.

The findings indicative of nervous system involvement usually associated with acute porphyrinuria are of motor nature. Peripheral neuritis and ascending paralysis of Landry's type are the most prominent clinical types. Mason, Courville, and Ziskind's monograph gives a complete clinical and pathologic description. Boulin, Garcin, Nepveux, and Ortolan[12] reported acute porphyrinuria with peripheral paralysis showing pathologic changes in the anterior root cells in the cervical and lumbar areas in the cord. There were no sensory symptoms in their patient. Waldenstrom's[13] monograph is a most complete summary of clinical, chemical, and pathologic findings. He studied 50 patients in only one of whom were there sensory changes. He states (loc. cit. p. 103): "Sensation is in great majority of cases entirely intact. Only by long presence of symptoms have I been able to observe undoubted appearance of superficial as well as deep disturbance of sensibility. . . . As a rule only the motor neuron is involved." In the case studied sensory symptoms were among the first complaints in the numbness in the feet. Objective evidence of loss of superficial and deep sensation below the twelfth dorsal segment and description of paresthesia in the hands were definite.

When the first reports on photosensitivity following treatment with sulfanilamide appeared, the thought occurred of disturbance of pigment metabolism. A case of meningitis treated with sulfanilamide and meningococcus antitoxin under observation concurrently with the case studied also showed porphyrinuria. Burnsting's[14] report spoke of porphyrinuria following sulfanilamide. Rimington and Hemmings'[15] clinical and chemical study showed that patients receiving sulfanilamide showed hematoporphyrinuria in amounts

nearly ten times that normally found, and that this continued even after the drug was stopped. The tabulation of clinical diagnoses in their article does not mention any central nervous system disorder.

Whether the use of gonococcic filtrate in the case studied was of any etiologic significance is quite speculative. A situation perhaps indirectly comparable is in the article by Marinesco, Dragenesco, and Chiser (loc. cit.) in which they described a myelitis of the herpes zoster type developing after seven injections of gonococcus vaccine. Their patient, however, had had poliomyelitis in boyhood and encephalitis in adult life and hence already had a damaged central nervous system. Gonococcus filtrate has been widely employed with many favorable and some unfavorable results reported. No central nervous system reactions have been seen described.

SUMMARY

A case of myelitis in a patient with gonorrhea has been described. The patient had taken sulfanilamide. The color of the urine suggested porphyrinuria which was confirmed by spectroscopic examination. Some of the possibilities of causation of the myelitis have been discussed with the conclusion that the diagnosis was gonorrheal myelitis with associated porphyrinuria following sulfanilamide.

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THE HEMATOLOGIC STUDY OF 76 PNEUMONIA CASES TREATED WITH SULFAPYRIDINE, INCLUDING A FATAL CASE OF AGRANULOCYTOSIS*

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THE introduction of chemotherapy in the treatment of pneumonia and other medical conditions naturally presents the problem of whether the drug in question has any toxic or other untoward effects. The use of sulfapyridine, M and B 693, or Dagenan, in the treatment of pneumonia was begun at the Toronto Western Hospital in October, 1938. At that time there was no literature concerning possible toxic effects of the drug upon the hematopoietic system, and we undertook this study. In the meantime, several reports on the use of sulfapyridine in the treatment of pneumonia and other infections have been published. The volume of this work is rapidly increasing, and a more complete knowledge of the biochemical reactions, clinical uses, and toxic effects of this chemotherapeutic agent is becoming available. No attempt has been made to present an exhaustive review of the literature of this subject, because the rapidity with which new knowledge of the drug is being published would quickly out-date any summary of this type.

METHOD

Leucocyte and erythrocyte counts and hemoglobin estimations were taken before the institution of sulfapyridine therapy. Occasionally this procedure was not possible, and the work was started a few hours after the first dose was given. These estimations were repeated daily for the duration of the therapy and every two to three days thereafter until the patient's discharge. From time to time, where indicated, blood films were taken and examined with special attention to the differential leucocyte count. The estimations of the red and white blood cells were done in the usual manner. The hemoglobin readings were made using the standard Sahli instrument, allowing thirty minutes for the completion of the acid hematin reaction and correcting the readings to a standard of 15.4 gm. hemoglobin for 100 per cent. Frequent duplicate estimations of leucocyte, erythrocyte, and hemoglobin levels were made by two workers in order to assure accuracy of results.

RESULTS

The series here recorded consists of all persons given sulfapyridine therapy after the scheme of investigation outlined above was established, and comprises 70 patients. Six of these had two separate courses of the drug because of a

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second attack of pneumonia, extension, empyema, etc. The hematologic record of these patients was followed with each course of therapy and is included in our results as separate cases. The pharmacologic and clinical aspects of the whole series of cases have been recorded by Brown, Thornton, and Wilson,[1] and Detweiler, Kinsey, Brown, and Feasby,[2] and submitted for publication separately.

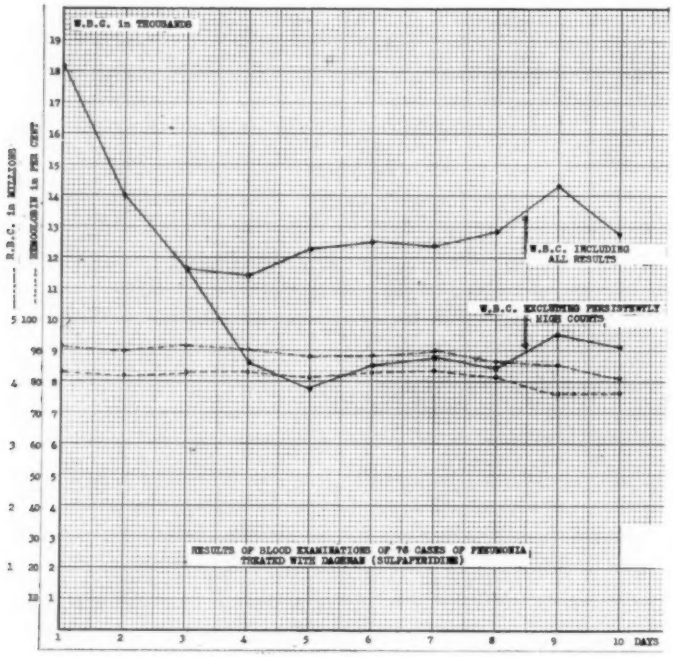
W.B.C. in THOUSANDS
R.B.C. in MILLIONS
HEMOGLOBIN in PER CENT
W.B.C. INCLUDING ALL RESULTS
W.B.C. EXCLUDING PERSISTENTLY HIGH COUNTS
RESULTS OF BLOOD EXAMINATIONS OF 78 CASES OF PNEUMONIA TREATED WITH DAGENAN (SULFAPYRIDINE)
DAYS

Chart 1.

In Chart 1 are recorded the results of the tabulation of blood examinations from the first to tenth day inclusive, showing the average levels of leucocyte, erythrocyte, and hemoglobin estimations. Study of this graph revealed a leucocyte fall during the first four days, with a slight elevation beginning on the fifth day and lasting to the tenth. The persistence of a leucocytosis of 12,000 or over occurred in 35, or nearly half, of the persons studied. The rest had levels within the normal range, as shown in the chart, throughout the remainder of their stay in the hospital. To determine the possible significance of the persistent leucocytosis, the 35 persons were separated for further analysis. Twelve of them showed no cause for leucocytosis, though 2 had had positive blood cultures, 2 had oliguria, 2 hematuria, and one had diabetes. The leucocyte levels, however, were not of a high nature, the maximum being 22,300. The remaining 23 patients showed a variety of complications and concomitant disease states as follows: simple empyema 4, simple effusion 2, effusion and pneumonic extension 1, effusion and delayed resolution 1, effusion and peripheral phlebitis 1, delayed resolution 2, pneumonic extension 1, extension and infective asthma 1, and pneumonic extension and empyema 1. The remainder of this group showed the following associated conditions: infective asthma, severe pulmonary tuber-

culosis, gonococcal arthritis, salpingitis, postgastroenterostomy and lung abscess, postcholecystectomy, carcinoma of breast, and ureteral calculus with probable kidney infection.

Though this series is admittedly small in number, it is thought to be of significant importance to stress the fact that of 35 patients with a persistently high leucocyte level after the third day of sulfapyridine therapy, 65 per cent presented a definite complicating factor, and of these, 46 per cent were of a pulmonary nature.

Examination of the average erythrocyte and hemoglobin levels showed, over the ten-day period, a decline of the former from 4,580,000 to 4,010,000, and of the latter, from 82.6 per cent to 76.6 per cent. Even without considering the factors of fever and dehydration, which produced elevated blood levels in the first two to three days, the total drop in erythrocyte and hemoglobin levels was not beyond what is anticipated in this type of acute infective processes. The maximum fall of hemoglobin occurred in a 16-year-old boy who developed effusion and empyema, and whose hemoglobin fell from 90 to 63 per cent in a nine-day period. The maximum fall of from 5,700,000 to 4,100,000 erythrocytes in ten days occurred in a 21-year-old man who also developed empyema. In these cases, fever and dehydration probably produced the initial elevated level of erythrocyte and hemoglobin estimations. Furthermore, in each of these cases the causative agent was a hemolytic streptococcus.

Apart from the foregoing results there were no hematologic observations which would indicate any tendency for the drug in question to cause a hemolytic type of anemia. One patient, J. R., recorded in detail in this study, received a dosage of 85 gm. of the drug over a period of 16.5 days, with no hemolysis occurring. Another patient receiving high dosage without developing anemia was H. F., a male, who was given 48 gm., in seven days, with an average daily intake of 6.9 gm. This patient had a short period of anuria during which the blood levels reached 32.6 mg. total and 16.6 mg. per 100 c.c. free drug.

Leucopenia was considered to be present when the leucocyte count fell to a level below 5,000. Of the 26 cases in this series, depression of the leucocytes below this number occurred in 8 patients in whom 27 low estimations were recorded. In only 2 cases was the leucocyte depression noted but once, while the most persistent leucopenia observed was one in which the condition was present on seven successive days. Blood-film examinations presented differential counts which varied with the degree of depression. When the total count was between four and five thousand cells, the lymphoid elements were relatively increased, but retained their absolute number. With a further decline of the leucocyte level, there was an absolute fall of both lymphoid and myeloid elements, with maintenance of the relative lymphocytosis. Detailed examination of the white blood cells revealed no significant changes in the lymphocytic or monocytic elements. The neutrophiles presented little change beyond a moderate shift to the left and an increase in toxic granularity. There were no forms seen which were less mature than the juvenile type. The erythrocytes revealed but little variation from normal, the only change noted being the appearance of an occasional polychromatic cell. Table I presents the most significant features of the patients who developed a leucopenic state.

TABLE I

PATIENT	AGE	SEX	LEUCOCYTE COUNTS		DAGENAN DOSAGE (GM.)			HIGHEST NOTED DAGENAN BLOOD LEVELS (MG. PER 100 C.C.)			CAUSATIVE ORGANISM
			ADMISSION	LOWEST	TOTAL	DAYS GIVEN	AV. DAILY DOSE	TOTAL	FREE	CONJ.	
T. W.	16	M	23,000	4,500	6.0*	1.0	6.0	6.9	6.7	0.2	Pneumococcus type II
M. B.	14	F	5,400	2,600	10.0	1.0	10.0		6.4		Pneumococcus†
V. B.	41	F	8,500	3,000	42.0	7.0	6.0	11.8	7.8	4.0	Pneumococcus†
B. M.	50	F	6,100	3,400	14.0	2.0	7.0		9.3		Pneumococcus type I
J. S.	47	M	18,600	4,500	22.5	3.5	6.3	17.6	14.4	3.2	Pneumococcus type IV
F. L.	32	F	25,400	2,800	30.0	6.0	5.0	5.4	3.4	2.0	Pneumococcus type VII
J. C.	27	M	5,300	3,300	16.0	2.5	6.4	10.4	9.5	0.9	Pneumococcus type VI
J. R.	59	M	7,700	700	85.0	16.5	5.1	26.3	13.8	12.5	Hemolytic streptococcus
Average			11,250	3,100	28.2	4.9	6.4	13.0	8.9	3.8	
Total Series Average					33.8	6.5	6.2	13.0	9.9	3.0	

*Intravenous soludagenan (sodium sulfapyridine) 2 doses of 3 gm. each.
†Pneumococcus did not type.

The histories of all 8 patients were carefully rechecked, and it was found that they all had typical signs and symptoms of pneumonia, with confirmatory x-ray examinations in several cases. The age range was from 16 to 59, and the cases were equally divided between the sexes. The initial leucocyte counts were low, only 3 being above 8,500. The totals of sulfapyridine dosage and the number of days the drug was given are lower than the average of the whole series, because with the onset of leucopenia, the drug was discontinued. The highest levels of drug concentration in the blood are comparable to those of the series as a whole, but were not maintained because of discontinuance of the drug. There was no predominating type of pneumococcus. In the case of the sputa which did not type, sulfapyridine had been given before the sputum was obtained for typing.

Agranulocytosis.—At the time of presenting this study, the medical literature contains reports of at least 4 cases of agranulocytosis following the use of sulfapyridine. These include the cases of Barnett and co-workers,[3] Johnston,[4] Coxon and Forbes,[5] and Sutherland.[6] We have had one case. All of these cases, as well as our own, received large doses of the drug, as shown in Table II.

TABLE II

CASE	DOSAGE IN GM.	DAYS GIVEN	AV. DAILY DOSE	ORGANISM
Barnett et al.	80.9	17.0	4.8	Pneumococcus type I
Johnston	54.0	11.0	4.9	Streptococcus viridans
Coxon and Forbes	54.0	17.0	3.2	B. typhosis
Sutherland	52.0	12.5	4.2	Streptococcus hemolyticus
Morgan	85.0	16.5	5.1	Streptococcus hemolyticus

In addition to the foregoing incidences of agranulocytosis, Tzanck and associates[7] report a case of agranulocytosis where only moderate doses of the

drug were employed, and we have knowledge of a further fatal case which occurred in another Toronto hospital. The most important features of our case of agranulocytosis are included in the following case report.

J. R., a male, aged 59 years, was admitted to the medical division March 9, 1939. He gave a history of asthma of two years' duration, and weakness, malaise, headache, and slight cough for one week. Examination revealed an acutely ill patient who was dyspneic and slightly cyanosed. Physical and x-ray examinations revealed pneumonia of the right upper lobe. The patient was given sulfapyridine therapy in the dosage recorded in Chart 2. The temperature fell to normal, and after six days' treatment, the sulfapyridine was discontinued. The following day the fever returned, and the use of the drug was resumed. During this course of the drug the patient developed a maculopapillary rash and oliguria, as noted on the chart.

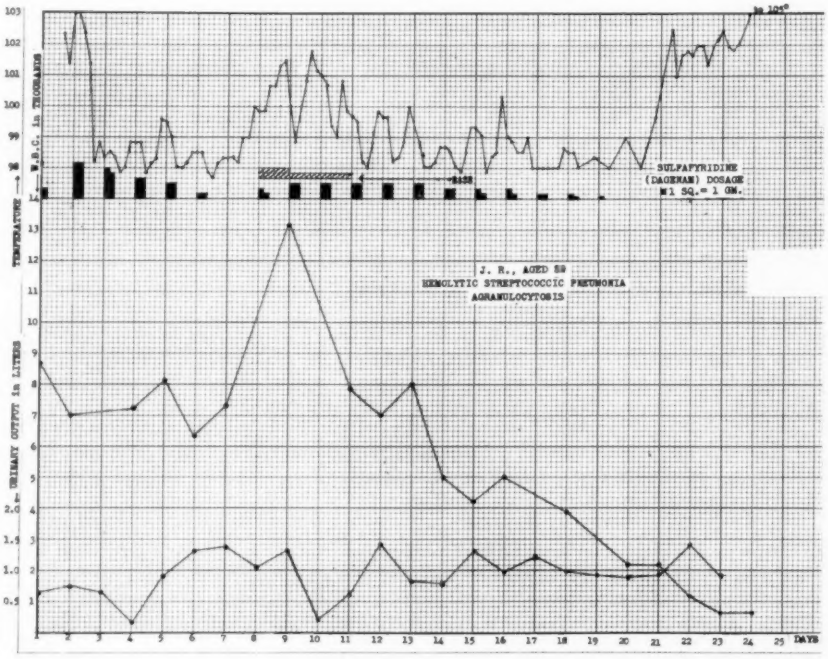

Chart 2.

After twelve days he had a normal temperature, felt well, and had no abnormal pulmonary findings, and the drug was discontinued. Two days later his fever again returned, and he had evidence of pneumonia in his left lower lobe. At this time his leucocytes numbered only 2,200, with 100 per cent lymphocytes. He was given repeated transfusions and daily intramuscular liver injections, but his leucocyte level continued to fall, and in three days his count was only 700. The pneumonic process spread to all lobes, and he died the twenty-third day after admission.

Bacteriologic examination revealed *Streptococcus hemolyticus* with good inhibition of growth in vitro in 5 and 10 mg. per cent sulfapyridine in broth. The essential features of the autopsy were as follows: Both pleura showed old dense adhesions over all surfaces, with fresh inflammatory reaction evident over the lower lobes; there were 200 c.c. of blood-stained fluid in the left pleural cavity. Macroscopically both lungs revealed a generalized pneumonic process in various stages. Microscopically the lung tissue, due to the absence of granulocytes, presented the appearance of a pulmonary edema rather than a pneumonic process. Marrow removed by sternal puncture immediately after death and from the femur and ribs at autopsy, showed a complete lack of granular cells. Several myeloblasts were present, but no evidence

of any maturation of these cells was found. There did not appear to be any change in the character of the erythrocytic series or arrest in their development. The thrombocytes were unaltered.

On admission the leucocyte count of the patient was only 8,700, and during his illness only one count was obtained above 9,000. There was no significant decline in hemoglobin or erythrocyte levels. He developed a rash ten days before his agranulocytosis. The total dosage of 85 gm. was given over a 16.5 day period, with an average of 5.1 gm. daily. The highest blood levels reached were 26.3 mg. per 100 per cent total, 13.8 free, and 12.5 conjugated. At this time he had a high level of the conjugated form, which is thought by some workers to be more toxic than the free. This phase of the series is fully covered in a paper by Brown, Thornton, and Wilson,[1] which is now in the process of publication. This patient received the second greatest total amount of the drug of any of this series, though his average daily intake was not as high as the average for the whole group. While his blood levels were quite high initially when the doses were larger, his later figures were comparatively low.

The small series of agranulocytosis here presented shows that large doses were given over a comparatively long period of time before onset of the fatal condition. Without attempting to present a complete analysis of these cases, it seems apparent that the type, virulence, and extent of bacterial infection are the factors which determine the high dosage required to provide adequate chemotherapy. That the drug may have definite toxic effects on the development of the granular leucocytes is obvious. The onset of leucopenia, as previously shown, is not of necessity related to the intake of the drug, but it seems that agranulocytosis may develop with doses above 50 gm. As further reports are published, it is probable that this condition will be found to develop with smaller total doses, since it appears that there must be, as revealed by our series of leucopenic cases, an individual susceptibility of certain patients to the toxic effects of the drug. It is hoped that with the accumulation of data, more definite knowledge of the toxic properties of sulfapyridine may be obtained. Until an improved method of determining deleterious effects on the neutrophile series is found, it is considered necessary to do daily leucocyte counts and discontinue the drug immediately upon the appearance of leucopenia.

SUMMARY

The number of cases included in this study is admittedly small, and it is probably inopportune to attempt to draw any concrete conclusions from a group of this size. It is hoped, however, that our results, taken in conjunction with those of other workers, will assist in revealing the possible toxic effects of the drug upon the hematopoietic system.

Of 35 patients with a persistent leucocytosis after the third day of sulfapyridine therapy, 65 per cent had a demonstrable complicating factor, and of these, 46 per cent were pulmonary in character.

Sulfapyridine did not produce any significant decline in hemoglobin or erythrocyte levels.

Leucopenia developed in 8 out of 76 cases, approximately 10 per cent. There was a relative lymphocytosis when the total leucocyte count fell to 4,000; below this level the cells of both the lymphocytic and granular series were reduced in total number, with the relative lymphocytosis being maintained. There were no significant changes in cell characteristics.

The leucopenia did not seem to be related to age, sex, causative organism, dosage, or blood levels of the drug. It appears evident that patients with low initial leucocyte counts are much more liable to leucopenia.

A case of sulfapyridine-treated hemolytic streptococcal pneumonia, which developed a fatal agranulocytosis, is reported in detail. It seems evident that, until more complete knowledge of the drug is obtained, daily hematologic examinations are necessary so that with the development of leucopenia, the drug may be immediately discontinued before agranulocytosis may supervene.

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A STABLE ADRENAL CORTICAL EXTRACT*

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ADRENAL cortical extracts have been prepared by various workers.[1-4] When freshly prepared, the extracts of Hartman and Swingle and Pfiffner were found successful in prolonging the life of adrenalectomized animals by various workers,[5-7] but the potency of these extracts is rapidly diminished even at icebox temperatures, so that by the end of two to three weeks these extracts are found to be ineffective. More recently, Cartland and Kuizenga[8] reported a method of preparing adrenal cortical extracts which they claim remain stable for longer periods.

This paper describes a method of preparing an adrenal cortical extract, which tends to retain its potency upon standing at room temperature for at least eighteen months.

METHOD

Whole adrenal glands from freshly slaughtered cattle, collected on the floor of the abattoir, are chilled in carbon dioxide ice and immediately brought to the laboratory where they are finely ground in a meat chopper. Each pound

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of gland is placed in one liter of 95 per cent alcohol and shaken in a mechanical shaker from sixteen to twenty hours, as originally described by Hartman.[9] The glands are then pressed in an ordinary wine press through a dish towel. The residue is discarded, though it may, however, be re-extracted with 80 per cent alcohol, using one liter per pound of gland, and again shaking as described above. The second extraction adds 10 to 15 per cent to the potency of the final extract. The alcoholic extracts are filtered and evaporated in vacuo at 45° C. (The insertion of a capillary tube, provided with a rubber tubing and a screw clamp into the rubber stopper, effectually prevents foaming during evaporation and also oxidizes the adrenalin. By adjusting the screw clamp, enough air is admitted to break up the films and yet maintain sufficient negative pressure to insure rapid distillation.) The aqueous sludge, which remains after the alcohol is evaporated, is washed with equal volumes of benzol until the last washing of benzol is colorless. Usually four to six washings are sufficient. The aqueous sludge is discarded. The benzol portion is evaporated in vacuo to a thick paste. The residue is dissolved in 70 per cent alcohol, using 100 c.c. of alcohol for each pound of original gland. The alcoholic extract is placed for at least twelve hours in an icebox 4 to 6° C. A heavy, dark precipitate is brought down, which can be separated by filtering in the icebox. This precipitate, which melts at room temperature, is very toxic to dogs and rats. The alcoholic filtrate is then evaporated in vacuo, and when the alcohol has evaporated, the aqueous portion is again washed with equal volumes of benzol until the benzol is colorless. Usually three to five washings are sufficient. The aqueous portion is discarded. The benzol is evaporated to dryness, and the residue is dissolved in 70 per cent alcohol, using 50 c.c. of alcohol for each pound of original gland; the alcohol is again placed overnight in the icebox. The process of washing the aqueous portion with benzol, and redissolving in 70 per cent alcohol and placing in the icebox as just described is repeated until no more precipitate is formed. When the precipitate is no longer formed from standing overnight in the icebox, the aqueous portion is washed with benzol, which in turn is evaporated to dryness. The residue is dissolved in 95 per cent alcohol (10 c.c. per pound of original gland) and this again is evaporated to dryness. The residue is extracted in 20 per cent alcohol for thirty to forty minutes. The amount of 20 per cent alcohol used in the last extraction may be varied according to the concentration one desires. The final extraction is passed through a Seitz filter. (The plates of the Seitz filter are alkaline, pH 10 to 11; therefore, it is best to wash the plates with 100 c.c. of 6 per cent acetic acid, and wash the acid out with 400 c.c. of water.) The final extract has a clear light yellow color.

The 20 per cent alcoholic extract is miscible with any volume of water or alcohol. In this work, enough alcohol was added to make it 50 per cent by volume and kept at room temperature from one to eighteen months. Before using the 50 per cent alcoholic extract, the extract was diluted to a concentration of 10 per cent alcohol; however, when the 20 per cent alcoholic extract is used, it may be injected without dilution.

The final extraction may be made with water, but in the aqueous state the extract is not stable.

CHEMICAL ANALYSIS

No detailed and exhaustive chemical analyses have been made on the extract.

The extract is free from nitrogen. The adrenalin content must be very low, for 5 c.c. of extract injected intravenously has no effect on the blood pressure of cats anesthetized with ether.

Eagle,[10] who has found considerable quantities of choline in adrenal cortical extracts prepared according to methods of other workers, found 1.8 and 1.2 gamma per c.c. of choline in two samples of this extract.

METHOD OF ASSAYING

Dogs, apparently normal, were adrenalectomized in two stages. The animals were kept on the same diet before and after surgery. The diet consisted of ground meat mixed with bread and bone meal (the standard diet used in this laboratory). The dogs were fed once a day. Water was always present in their cages.

Subcutaneous injections of extract were started on the second day after the second adrenal gland was removed. It was found that dogs survived better under this regime than they did when the extract was started when definite symptoms of adrenal insufficiency were in evidence. The injections of the extract were continued ten to twelve days and then withdrawn. When symptoms of adrenal insufficiency set in, the administration of the extract was resumed and continued until the animal appeared normal. Again the extract was withdrawn. When the dog showed signs of adrenal insufficiency, injections of the extract were again resumed and continued until the animal was apparently normal. Final withdrawal of extract led to speedy exitus.

A necropsy was performed on each dog. Macroscopic examination never revealed any remains of adrenal cortical tissue.

An effective dose was considered one which would keep the animal not only alive, but obviously in good physical condition. It was found that not every batch of glands extracted yielded the same potency on the same dog; nor were the requirements of each dog per kilogram of body weight the same. For example, Dog 30, weighing 9.4 kg., required 5 c.c. of extract 128, while Dog 4, weighing 12 kg., required 10 c.c. of the same extract to keep it in good condition. Using extract 131, Dog 30 required 10 c.c. per day to keep it in good condition, in contrast to 5 c.c. of extract 128. Both extracts represented the same amount of whole adrenal gland per cubic centimeter of extract, namely, 45 gm. of fresh gland.

In all, 29 dogs were used in this work, 10 of which were used as controls to note the survival period of bilaterally adrenalectomized dogs without therapy, and 19 were treated with extract. The survival period of the control dogs was, on the average, four days. The longest survival period was five days, and the shortest, two days. The treated animals were permitted

to live on the average for twenty-three days. The longest period a treated dog was permitted to live was thirty-two days, and the shortest period, twenty-one days. In this group, each dog had adrenal insufficiency at least twice and recovered with extract therapy. Final withdrawal of extract caused death within four days.

Following is a protocol of a typical experiment.

Dec. 21, Removed right adrenal.
Feb. 9, Removed left adrenal.
Feb. 11, Dog did not stand up and did not eat. Injected 10 c.c. extract 107B.
Feb. 12, Dog stood up and ate its food. Injected 10 c.c. of 107B.
Feb. 13, Dog was in fine condition. Injected 8 c.c. of 107B.
Feb. 14, Dog was in fine condition; ran around and was very active. Injected 8 c.c. of 107B.
Feb. 15, Dog was in fine condition. Injected 12 c.c. of 107B2.
Feb. 16, Dog was in fine condition. Injected 12 c.c. of 128.
Feb. 17, Dog was in fine condition. Injected 10 c.c. of 128.
Feb. 18, Dog was in fine condition. Injected 6 c.c. of 128.
Feb. 19, Dog was in fair condition. It was not as active as on previous days. Injected 10 c.c. of 128.
Feb. 20, Dog was in good condition, and was again very active. Injected 10 c.c. of 128.
Feb. 21, Dog was in fine condition. Injected 10 c.c. of 128. This dog was among other dogs and a fight ensued in which this dog also participated. In the evening it was very weak. Injected an additional 10 c.c. of 128.
Feb. 22, Dog was able to run around and appeared to be in good condition. Injected 6 c.c. of 128.
Feb. 23, Dog was in fair condition. No extract administered.
Feb. 24, Dog was in fairly good condition, did not eat all of its food, and vomited. No extract was administered.
Feb. 25, In the morning the dog was able to walk around. In the evening its gait was staggering and it was unable to climb into its cage. No extract was administered.
Feb.26, At 6 A.M. the dog was unable to stand up; it vomited the little food it had eaten. Injected 15 c.c. of extract 128. At 12:40 P.M. the dog was able to rise and walk a little. Injected an additional 5 c.c. of 128. At 8 P.M. the dog was able to run around, appeared to be in excellent condition, and ate its food.
Feb. 27, Dog was in excellent condition. Injected 5 c.c. of 128.
Feb. 28, In the morning the dog was in excellent condition, but in the evening it was very lethargic. No extract was administered.
March 1, Dog had a staggering gait so typical of adrenal insufficiency. Injected 9 c.c. of extract 109BHO.
March 2, Dog was in excellent condition. No extract was administered.
March 3, Dog was lethargic and refused to eat. No extract was administered.
March 4, In the morning the dog was unable to walk; it was very unresponsive all day; in the evening it had convulsions.
March 5, Dog died in the morning.

At necropsy no visible adrenal cortical tissue was found. The gut was hyperemic, but otherwise there were no visible changes.

DISCUSSION

The method here outlined differs essentially from other methods of preparing adrenal cortical extract, by the use of only two extractives, alcohol and benzol (both being relatively inexpensive). The laborious method of removing adrenalin by adsorption to permutit of Swingle and Pfiffner[11] is eliminated. The shaking during the extraction and the admission of air during the evaporation oxidizes the adrenalin.

Almost any lipoid solvent may be substituted for the alcohol and benzol; there is no advantage as far as potency is concerned, and the cost of most other solvents, such as acetone, chloroform, and ether, is greater. Although the final extraction is made with alcohol, the potent alcoholic extract can be diluted with water or physiologic saline in all proportions; thus, one may avoid any damage to tissues following injections. Repeated subcutaneous injections of 20 per cent alcohol in dogs caused no demonstrable deleterious effects.

Although other workers[1-7] kept adrenalectomized dogs and cats alive for longer periods than we did, the repeated symptoms of adrenal insufficiency which occurred when the extract was withdrawn and the recovery upon resumption of administration of extract show that the extract is able to keep adrenalectomized animals alive. There is no reason to doubt that dogs would not have lived indefinitely were the administration of extract continued.

SUMMARY

A new method of preparing adrenal cortical extract, which is stable for at least eighteen months and free from protein, is described.

I herewith wish to thank Dr. Carlson for his constant help and pertinent advice throughout this work.

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PERSISTENCE OF INFECTION WITH GIARDIA INTESTINALIS IN MAN*

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HUMAN infections with the intestinal flagellate, *Giardia intestinalis,* are of somewhat common occurrence, and are occasionally observed under circumstances which strongly suggest that the parasite is responsible for disease in the small intestine or gall bladder. As yet, however, the causal relationship of *Giardia* to either pathology or symptoms in man has not been established. Often the parasite is difficult to eliminate from one who harbors it, and infections may persist in the presence or absence of symptoms for long periods. Such a case of prolonged infection, with occasionally distressing symptoms, has come to our attention, and, in the present paper, we shall present some significant points of the history and other facts concerning it.

History.—When the infection was first determined, the patient was a student in a parasitology class at the University of California, Los Angeles. She was suffering from a severe diarrhea during March of that year (1934) and, on examining her own stool, observed both motile forms and cysts of *Giardia intestinalis.* Her observations and identifications were corroborated by the instructor of the class, Dr. Gordon Ball. About eighteen months later, during the summer of 1936, the patient suffered a very distressing intestinal upset lasting six weeks, with blood appearing in the diarrheic stools. In spite of careful study and search by the attendant physician, no specific cause for the symptoms could be found, although *Giardia* was again proved to be present. Carbarsone was administered as treatment to eliminate the protozoan, and, although the number of parasites was temporarily reduced thereafter, the forms never entirely disappeared and soon became as numerous as they had been originally. Eventually the intestinal condition improved, although no specific reason for the improvement could be ascertained. A few months later the patient moved to New York City, and in February, 1937, we found both trophozoites and cysts of *Giardia.* In January, 1938, and again in February, 1939, we again observed the cysts of the parasite in the formed stools of the patient. They were numerous, and unquestionably cysts of *Giardia,* as judged by examination of both a fresh saline emulsion of the feces and an emulsion to which iodine had been added.

Symptoms.—The patient occasionally suffers from intestinal disturbances, with more or less severe diarrhea and epigastric pain before breakfast or between meals during the day. The attacks are most intense in the spring and summer months. Sometimes blood appears in the stools. At such times, motile specimens of *Giardia* can be observed in the stools, although between such attacks, *Giardia* cysts alone have been found. The motile forms can be brought forth at will, nevertheless, by the administration of a purgative.

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COMMENT

It is neither assumed nor suggested that *Giardia intestinalis* is the cause of the symptoms described in the case presented. Wenyon has pointed out that "blood never occurs in pure *Giardia* infections," and blood was found in the stools of this patient during the periods of intense diarrhea. Although no other agent which might cause this condition has been determined, we, nevertheless, leave open the question of the causal relationship of the parasite. This case has been reported only because of the long persistence of proved infection with the parasite, *Giardia intestinalis*.

It is not definitely known whether the patient represents a single prolonged infection or a succession of reinfections, although there seems greater likelihood for the former. This is indicated not only by the fact that the patient is of the upper social strata and lives under good hygienic and sanitary conditions, but also by the infection persisting after a major geographic shift—from the West to the East Coast. If it is true that this case does represent a single prolonged infection, then there appears to be a distinct difference between the duration of infection in this case with *Giardia intestinalis* and the infection of rats with *Giardia muris*. Hegner has demonstrated experimentally that rats lose their *Giardia* infections in about five months if reinfection is prevented. In his experiments, 20 rats kept in cages with a screen bottom so that fecal pellets fell through were free of their original infection in about five months. Nineteen of 20 control rats kept in a cage with shavings were found still infected after this interval. In contrast with this result in rats, the present case has harbored the human parasite for over five years and shows great promise of continuing to do so for an indefinite period.

SUMMARY

A case is reported of prolonged infection of a patient with *Giardia intestinalis*, the parasite having been observed in the stools at intervals over a period of five years. It is noted that the persistence of this parasite in man may be distinctly greater than that of a related form, *Giardia muris*, in rats, since rats are found experimentally to lose their infection within five months if reinfection is prevented.

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LABORATORY METHODS

THE DETERMINATION OF UREA IN BLOOD AND URINE BY CONWAY UNITS*

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SELJESKOG and Cavett[1] described the methods commonly used for the determination of urea in blood and compared them as to accuracy and simplicity in the November, 1937, issue of this JOURNAL. Although most of these methods gave efficient results, I would like to draw attention to a method which may be called the "Columbus egg" of the methods for the determination of urea. It is the modified method of Conway.[2]

For analyses on a large scale the Conway method has many advantages over other methods: (1) Only 0.2 c.c. blood or plasma are required for one estimation. (2) This method is the most accurate of all methods and is about twice as exact as Van Slyke's manometric procedure B.[3] If 0.5 c.c. blood are used, even Kirk's[4] very exact results can be exceeded in accuracy, and it is possible to determine the blood urea concentration with an accuracy of about 0.07 mg. per cent. (3) A large number of determinations can conveniently be accomplished simultaneously, the average time taken for one estimation being about four to six minutes. By suitable arrangement the action of the urease causes no loss of time at all. The diffusion proceeds without any supervision. (4) By Abelin's[5] modification the standard mineral acid for the reception of the ammonia is replaced by boric acid, and the ammonium borate is titrated directly with a standard mineral acid (viz., Brecher[6]). The standard alkali solution is replaced thus by the stable standard acid. The relatively strong boric acid in the receiver absorbs also abnormally high amounts of ammonia and, therefore, a special procedure for high urea values is unnecessary. For the same reason the estimation of urea in urine can be made with the same solutions, and the analyses of blood and urine can be performed simultaneously. (5) If only the U:P ratio and not the absolute urea values are required, the titration can be made with a nonstandardized acid, the U:P ratio being calculated by simple division of the titration values. (6) This method can be learned easily and makes clean and quiet working possible. The expense involved by the method is limited to not much more than the purchase of the Conway units, the cost of the reagents being very modest.

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It is obvious that a method with these advantages of accuracy and velocity is very suitable for scientific work and for everyday laboratory practice. In our clinic we have used this method for two years with the most satisfactory results for the determination of the urea clearance, and have saved much time in so doing. The introduction of the boric acid as an absorbing acid is a further step in the simplification of the method.

PRINCIPLE

The principle of the Conway unit* consists in the diffusion of the ammonia formed from urea and liberated in the outer chamber of the very simple apparatus into the boric acid absorption solution in the inner chamber. Under the prescribed conditions the absorption is completed within one hour at 38° C. The ammonium borate is directly titrated with sulfuric or hydrochloric acid with a Conway microburette.[8] For the exact measures and figures of these apparatus see Conway's original papers.[2, 8]

REAGENTS

1. The fixative for the glass lid: 40 to 50 gm. solid paraffin are melted with 80 c.c. liquid paraffin.

2. One per cent boric acid in distilled water.†

3. Mixed indicator: 1 part 0.2 per cent methyl red in alcohol and 1 part 0.1 per cent methylene blue in alcohol.

4. Urease solution: Per unit 20 mg. highly active urease (Arlco urease) are dissolved in 0.5 c.c. water and 0.02 c.c. of the phosphate solution is added. The amount required is freshly prepared every day. In our practice the urease solution in 50 per cent glycerol (Sabine[10]) showed ammonia formation after some days. The phosphate solution contains 6.9 gm. $NaH_2PO_4 + 2H_2O$ and 17.9 gm. $Na_2HPO_4 + 12H_2O$.

5. A saturated potassium carbonate solution is boiled vigorously for twenty minutes, and the evaporated water is replaced. On cooling, crystals should be formed.

6. 0.04 N sulfuric acid in carbon dioxide—free water. Cleaning of the units: After use the units are washed in a hot water current with soap and a hand brush; they are then stored in a weak acid containing some indicator. Before use they are washed several times with distilled water and are left to dry in the air.

PROCEDURE

The required number of units and two more for the blank analyses are dried. The glass lids are smeared with fixative. A sufficient amount of the boric acid is boiled for one minute to drive out the carbonic acid, and one drop of the indicator solution is added per cubic centimeter of solution. One cubic centimeter of the warm acid is put into the inner chamber of each

*The original pyrex glass Conway unit is available from A. Gallenkamp, Finsbury Square, London. Borsook[7] recommends locally made units of glazed porcelain.

†One per cent boric acid is used instead of the 2 per cent proposed by Abelin.[5] The color change of the indicator in 2 per cent boric acid is not very sensitive, as in this concentration stronger polyboric acids are present (Kolthoff[9]).

unit, and the lid is closed. The color of the solution must be the same in all units and very near to the changing point of the indicator. The lid is displaced horizontally and 0.2 c.c. of blood or plasma is delivered from an Ostwald pipette into the outer chamber, then the lid is closed again. One-half cubic centimeter of the urease solution is now added in the same way into the outer chamber, the cover lid closed and the unit gently rotated several times to mix the blood and the urease solution. The action of the urease is completed in one minute. When the urease is added to the fourth unit, the action of the urease is already completed in the first unit, so that it is not necessary to spend time waiting for the fermentation.

Fig. 1.

The unit is now slightly tilted to collect the fluid at one side of the chamber. The cover is now displaced so far as to allow the introduction of 1 c.c. of the potassium carbonate into the opposite side of the outer chamber. The carbonate is introduced with a pipette with a rather wide opening, an error of 10 per cent in the fluid volume being without any effect. An automatic pipette is most practicable in delivering the carbonate. After quickly replacing the lid, the unit is gently rotated ten times and then placed in an incubator at 38° C. for one hour or left at room temperature for one hour and a half. Care must be taken that the lid does not move during the placing into the incubator. The blank analyses are treated in the same manner.

Five cubic centimeters of ten times diluted urine are shaken for five minutes with 1 gm. permutit. Two-tenths cubic centimeter of the supernatant urine dilution are taken for analysis and treated exactly in the same way as the blood.

The color of the indicator changes to green during the period of the diffusion. The titration is carried out most conveniently with the Conway microburette.[8] The tip of the burette is emerged during the titration. The titration is continued until the red color does not grow any darker. With a little practice this color end point is found easily and the titration values of double determinations check within 0 to 1 mm. of the burette graduation.

CALCULATION

A is the number of millimeters of 0.04 normal sulfuric acid required to titrate the unknown,

B is the millimeters required to titrate the blank.

$1 mm^3$. 0.04 N H_2SO_4 = 0.28 mg. per cent urea nitrogen in blood
$1 mm^3$. 0.04 N H_2SO_4 = 2.80 mg. per cent urea nitrogen in urine

(1) If it is known that 1 mm. of the burette corresponds to x mm^3. of sulfuric acid, the following calculation is made:

$$(A - B) \times x \times 0.28 = \text{mg. per cent urea nitrogen in blood}$$
$$(A - B) \times x \times 2.80 = \text{mg. per cent urea nitrogen in urine}$$

(2) If the volume of 1 mm. of the burette is not known, 500 mm. of the sulfuric acid are measured and titrated iodometrically with y c.c. 0.005 normal thiosulfate solution.

$$(A - B) \times y \times 0.07 = \text{mg. per cent urea nitrogen in blood}$$
$$(A - B) \times y \times 0.7 = \text{mg. per cent urea nitrogen in urine}$$

(3) The U:P ratio is $\frac{10\ (A - B)\ \text{urine}}{(A - B)\ \text{blood}}$

SUMMARY

The advantages of the determination of urea by the Conway unit are summarized and the technique is briefly described. It is a micromethod with very great accuracy, and a large number of analyses can be done simultaneously, the average time for one estimation being only four to six minutes.

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A PRACTICAL THERMOELECTRIC THERMOMETER FOR CLINICAL MEASUREMENT OF SKIN SURFACE TEMPERATURES*

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UNTIL recently the clinical measurement of skin surface temperature has been relegated to the group of tests utilized comparatively rarely. Whether this was due to inconvenient or inaccurate methods, or to a seeming lack of importance, cannot be said, but it is becoming increasingly evident that determination of skin temperatures often facilitates diagnosis and charting the progress of certain diseases.

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Physical measurements, such as these, are important in the treatment of thromboangiitis obliterans, vasomotor neuroses, such as Raynaud's disease (Brown[1, 2]), arteriovenous fistula, peripheral embolism, and inflammations of the extremities. The beneficial effects of elevation and rigid immobilization in plaster casts of inflamed extremities, the skin temperatures being one of the indices of healing, have been summarized by Wangensteen.[7, 8]

SKIN TEMPERATURE MEASUREMENT

Seebeck in 1821 noticed that if a circuit is formed composed of two wires of different metals joined together at their ends, and if the junctions were at different temperatures, a thermoelectric current, proportional (to a certain point) to the difference in temperatures of the junctions, would flow in the circuit and could be measured by an interposed galvanometer. Kunkel[4] apparently was the first to use a thermocouple for measurement of human skin temperature.

Since the bulb expansion type of thermometer has been largely discarded for skin surface temperature measurement, the electrical resistance thermometer (Soderstrom[6]) and the radiometer (Hardy[3]) have been subjected to excellent refinements, but the thermocouple (Sheard and Williams[5]) retains the advantage of convenience, especially when used with one of the bronze wire double suspension galvanometers having sufficient sensitivity for use with thermocouple currents but being rugged enough to be carried about without locking the rotor.

Generally, contact methods of measuring skin temperature are inherently inaccurate because: (1) heat conduction between the thermometer and contiguous skin area will alter the reading unless the thermometer tip and skin are at the same temperature at the moment of contact; (2) the environmental air temperature affects the temperature of the uncovered contact tip; and (3) covering of the contact tip by a shielding or insulating material alters the heat equilibrium of the covered skin, changing the actual temperature at that point.

The above sources of error are held to a minimum by a properly designed skin thermometer having sufficient accuracy for clinical observation, if not for exact quantitative measurements, by (1) keeping the mass of the contact tip very low so conduction error is negligible; (2) using an anaerobic tip in contact with the skin; and (3) employing a thermometer designed with a very low lag; namely, one which gives an almost instantaneous reading, permitting movement of the tip along the skin, maintaining its contact.

In the routine measurement of skin surface temperature for clinical purposes in the University Hospital, we have used a portable thermocouple and galvanometer, accurately calibrated, which comes nearest to fulfilling the above requirements, the galvanometer reacting almost instantaneously to temperature changes in the tip. The apparatus, which is much more sensitive than clinical use necessitates, is compactly fitted into a box which is conveniently carried about by hand (Fig. 1).

The simple thermocouple junction applicator diagrammed in Fig. 2 has adequately met the technical conditions outlined and has proved highly satisfactory in diagnostic work. It consists of a piece of pernambuco or balsa

(any highly cellular substance would be adequate), measuring 8 by 1.5 by 1.5 cm. One end is beveled to permit its being applied at an angle, and a hole 4 cm. in depth of sufficient diameter just to accommodate the rubber sheath for the thermocouple wires is bored from the rectangular surface to the beveled end.

Fig. 1.—Portable thermoelectric skin temperature cabinet, showing manner of applying warm junction.

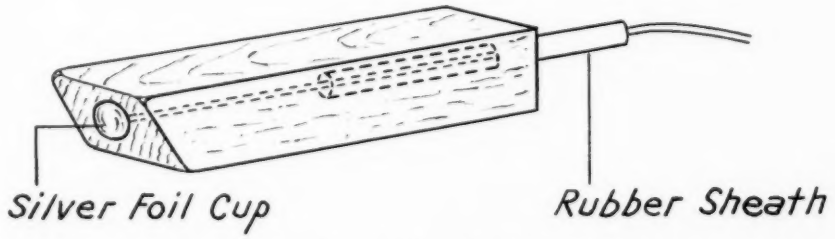

Fig. 2.—Diagram of warm junction applicator.

The previously soldered tips of the thermocouple wires (2 36G copper and 4 36G constantan) are threaded on a Keith abdominal needle and carried through the tunnel and remaining 4 cm. of wood to the beveled end. The concave surface of a pounded silver foil cup, 4 mm. in diameter and 1 mm. deep, is soldered lightly to the wire junction, and the wires are drawn taut to bring the cup snugly against the end of the applicator. By making the tip of such negligible mass, temperature equilibrium is attained almost instantly, the convex surface of the tip permitting its rapid movement along the skin from point to point.

The rate of equilibrium with room air at 27° C. of the warm junction after being exposed to a skin temperature of 34° C. is plotted in Fig. 3, galvanometer readings being taken every ten seconds. It will be seen that an exponential form of curve is obtained, a drop of over 3 degrees being obtained in the first ten seconds, thermal equilibrium having obtained in sixty seconds. For ordinary skin temperature measurements, where adjacent areas vary within 2 or 3 degrees, readings may be taken within five seconds after the junction is applied. Elevation of the warm junction temperature from a room temperature of 27° C. to a skin surface temperature of 34° C. takes place within ten seconds.

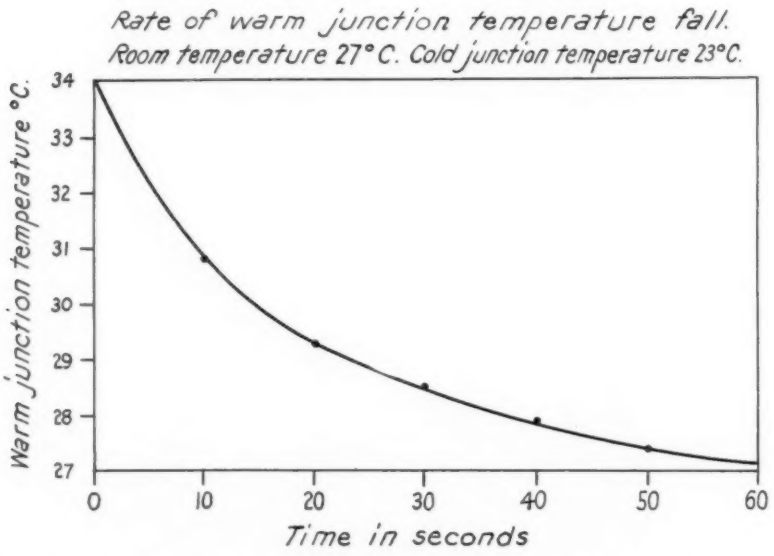

Fig. 3.—Rate of warm junction temperature fall after application to skin at 34° C. Room temperature 27°.

SUMMARY AND CONCLUSIONS

The importance of skin surface temperature measurements in certain surgical conditions has been pointed out. Data so obtained are helpful in diagnosis and evaluation of treatment in thromboangiitis obliterans, neurovascular diseases, arteriovenous fistula, embolism, and inflammations.

A new form of warm junction applicator for use with a handy, portable thermoelectric thermometer is described for routine clinical determinations of skin surface temperatures, the device being offered to meet the technical objections attending the use of contact methods.

Grateful appreciation is extended to Dr. Irwin Vigness, of the Department of Radiology, whose interest and cooperation was largely responsible for the construction of the apparatus.

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PHOTOELECTRIC PLETHYSMOGRAPHY OF ANIMAL TISSUES*

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SEVERAL photoelectric plethysmographs have been described for the study of vascular reactions in the animal, notably in the rabbit's ear.[1, 2] The application of the idea of photoelectric plethysmography to the study of the peripheral circulation in man[3-5] met with such success that it seemed worth while to explore further its possibilities in the animal. The technique described below was suggested by the method developed for the nasal septum.[3] It has the advantages of extremely high sensitivity, freedom from inertia and consequent distortion, ease of application of the plethysmograph, and relief from the irritation and errors associated with the mechanical type of plethysmograph.

The validity of the photoelectric plethysmogram and the sources of error involved have been discussed in previous papers.[4, 5]

Fig. 1 shows an arrangement of the photoelectric plethysmograph having a wide application in animal experiments. The tissue, whose vascular reactions are to be studied, is transilluminated by a pencil-type flashlight bulb. It is best to operate the bulb slightly below its rated voltage on an accumulator, to avoid a slowly shifting illumination intensity due to bulb deterioration or falling battery voltage. Either of these shows up in the plethysmogram as a gradual increase in the apparent opacity of the tissue. Light which has escaped absorption in the tissue is reflected into the photocell housing by the mirror placed at the end of the tube. Although the photocell may be placed directly beneath the tissue in some instances, the use of a mirror adds greatly to the convenience and flexibility of the technique. By using interchangeable tubes varying in diameter, the mechanical requirements of different situations may be conveniently served.

Semiquantitation of the plethysmogram is provided by comparing the changes in the plethysmogram with the deflection resulting from absorbing

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a fraction of the light with a filter. This is simply a sheet of thin glass (cover slip for microscope slides) on the filter carrier. During the recording of the plethysmogram, the filter carrier is quickly flipped into place, thus inserting the filter in the light path. The validity of this procedure has been discussed previously.[4, 5] Quantitation is also provided by the grid bias control described below.

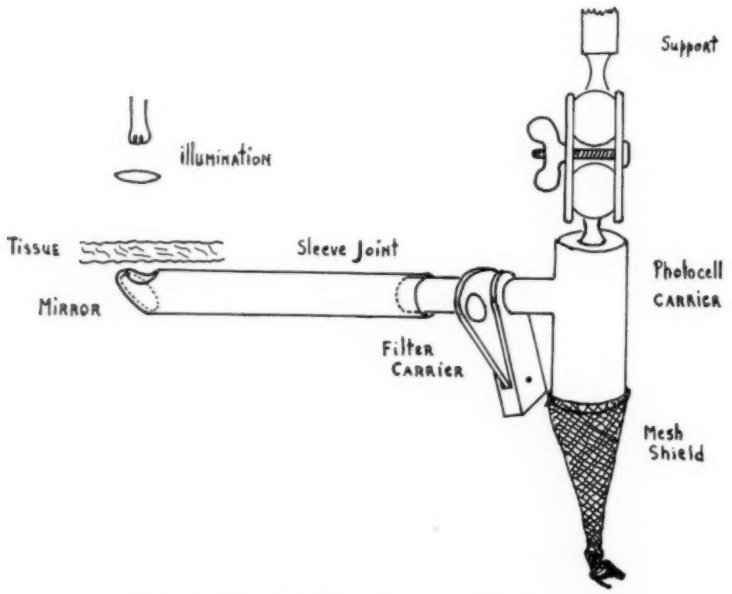

Fig. 1.—Photoelectric plethysmograph for animal tissues.

Technical Data on Amplification.—Although the use of amplification complicates photoelectric plethysmography, there are good reasons for employing it. Amplification eliminates the need for intense illumination with its possible heating effects and makes possible the use of high frequency, low sensitivity recording instruments. A suitable amplifier must be free from drift, and provide linearity of response as well as a wide range of amplification. The amplifier meeting these requirements most satisfactorily is a modification by Dr. H. B. Peugnet, of the standard two-stage direct coupled type (Fig. 2), employing 6SJ7 and 1852 tubes and delivering 0.35 Ma. per millivolt at full sensitivity, a gain which allows adequate variations in recording amplification. When these high Mu tubes are operated at less than their rated maximum plate and screen grid voltages, drift is greatly reduced. Further reduction of drift is obtained by eliminating thermocouple effects on the bias of the first tube by building it in a separate cabinet, connected to the amplifier proper by means of a Banana Jack. Further stability is secured after the tubes have been in use for several days due to the smoothing of the cathode with use, giving more constant emission of electrons. The cabinets are galvanized iron; all leads are shielded cable. Both are grounded. By allowing the amplifier to heat for at least an hour to reach thermal equilibrium, drift is less than 4 mm. per hour on the record when the sensitivity is sufficient to give a volume

pulse wave of 1 cm. As has been indicated above, drift on the record may be due to deterioration in illumination which shows up as apparently increasing opacity.

The amplifier is set by balancing the second stage by means of its grid bias *(GB2)* with the volume control switch *(VC)* off until the plate current reads zero. The volume control switch is then turned on, and the balancing is repeated by means of the grid bias of the first stage *(GB1)*, the volume control being set at any desired amplification. The amplification will follow accurately the curve of the volume control potentiometer and calibration is therefore possible. However, it is usually necessary to readjust the bias of the first stage when changing amplification, particularly if the change is large.

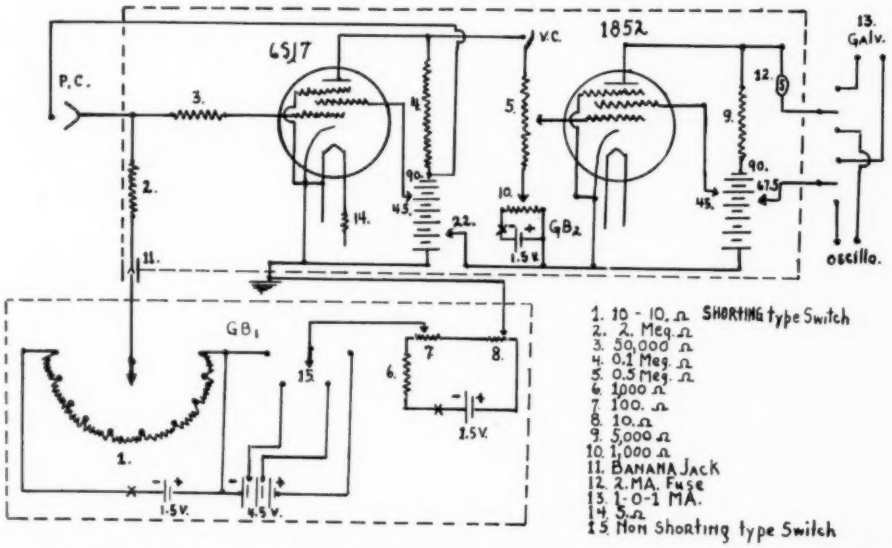

Fig. 2.—Two-stage direct-coupled amplifier for photoelectric plethysmography. *P.C.* Photoelectric cell, cathode to grid. GB_1, GB_2. Grid bias controls for first and second stages. Oscillo-oscillograph or string galvanometer.

The grid bias control on the first stage serves as an indicator of the input from the photocell and, therefore, of the changes in the intensity of the light reaching the photocell. This fact may be used instead of the filter to calibrate the plethysmogram in arbitrary units. Thus, by adjusting the volume control until a unit change in the input produces a unit deflection in the plethysmogram, several simultaneously recorded plethysmograms may be compared with respect to the size of the vascular changes.

This amplifier may be used with recording galvanometers, such as the General Electric type A, G6 or G7, Westinghouse 877-121, or the Hindle string galvanometer. The latter is not as satisfactory as galvanometers with fixed sensitivities, since the string tension and, therefore, the sensitivity, may change without notice. Further, the string galvanometer requires shielding or it serves as a feed-back of alternating current into the system. It is important to note that the shunt resistance 9 on the plate circuit of the second stage should have approximately the same value as the recording instrument.

Photoemissive cells, similar to the G. M. Laboratories 51A, are suitable. The small cells are to be preferred because of ease in manipulation. It is very important to shield the photocell. This is done by means of a galvanized iron mesh cone which fits well up on the photocell carrier which is also made of galvanized iron. Induction difficulties are thus avoided.

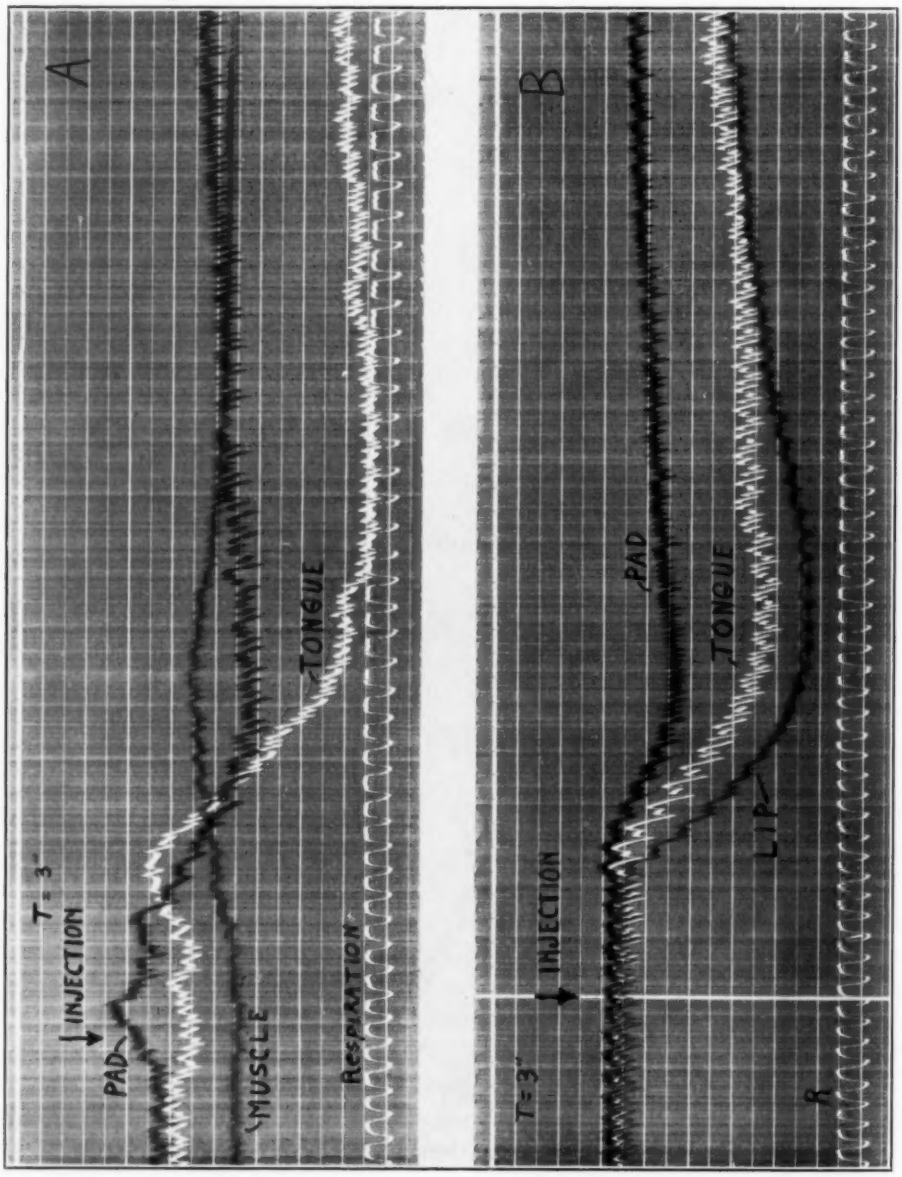

Fig. 3.—Photoelectric plethysmograms. *A*. Of thigh muscle, tongue and foot pad, 1 gamma adrenalin per kilogram intravenously. *B*. Of lip, tongue and foot pad, 0.5 gamma adrenalin per kilogram intravenously.

Illustrative plethysmograms are provided in Fig. 3, showing the effects of intravenous injections of adrenalin on the vascular areas in the tongue, lip, thigh muscle, and foot pad of the dog. Vascular changes in the latter area were recorded with the skin plethysmograph[5], those in the tongue, lip, and thigh muscle with the technique previously described. Well-known constrictor

and dilator actions of adrenalin are seen in these responses. It is interesting to note the strong constrictor action in the tongue despite the presence of striated muscle. This may be related to dominant effects on the mucosa and on arteriovenous anastomoses which have been found in the dog's tongue.

SUMMARY

A description of the application of photoelectric plethsymography to animal tissues is given. A suitable amplifier is designed which meets the requirements of freedom from drift, linearity, and wide range of amplification. The technique has adequate sensitivity for the study of vascular reactions in such limited areas as the tongue, lip, eyelid, skeletal muscle, etc. It is likewise applicable to arteriography.

We are greatly indebted to Dr. H. B. Peugnet, of the Washington University School of Medicine, for important aid in designing the amplifier described in the paper.

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A METHOD OF EMPLOYING HORSE PLASMA AND HEMOGLOBIN AS ENRICHMENTS IN PRIMARY GONOCOCCUS ISOLATIONS*

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EVER since Neisser first observed the gonococcus in 1879, many investigators have taken up the problem of a suitable medium on which the gonococcus would grow consistently on first culture from the human body. It was not until 1885 that Bumm[1] succeeded in growing and isolating the organism on coagulated human blood serum. Wertheim,[2] deciding that the growth of the gonococcus was tied up with human blood albumin, first produced a serum agar. He emulsified his culture material in human serum, diluted this emulsion with more serum, then mixing this with melted agar cooled to 40° C., he poured plates. Kiefer[3] brought the culture method closer to present-day procedures by first pouring the serum plates and then inoculating them with the culture material.

It was soon discovered, however, that serum did not give dependable results, and other sources of albumin were sought. It was then that ascitic and hydrocele fluids were tried. These were also found to be of uncertain

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value. Ascitic fluid, to be of service, must be bile free, of high specific gravity, and sterile. When contaminated, which it often is, it is difficult to sterilize, since filtering it removes most of its growth-inducing substances and heat coagulates the albumins. It also has a tendency to grow more alkaline and to deteriorate on standing. Abel,[4] therefore, went back to the use of blood, employing Pfeiffer influenza plates for gonococcus isolations. At first, human blood only was used, then rabbit blood, and finally Koch[5] showed that horse blood also was suitable.

Many and various media were then elaborated and tried, and the advantages of serum and red blood cells, as enrichments, were studied. It was found that red blood cells stimulated initial growth of gonococcus and that the presence of serum produced more luxuriant growths (Cole and Lloyd[6]). Animal tissues, such as beef heart and steak, were then substituted. But since in the preparation of the medium a large part of the necessary growth elements were lost because of heating and filtration, these media required other enrichments, such as egg (Huntoon[7]) or blood.

About 1920, the Levinthal medium, originally used for the influenza bacillus, came to be considered by many the most reliable medium for initial gonococcus isolations. This clear and colorless cooked blood agar was employed by Galli-Valerio,[8] by Cohn, by Strempel,[9] and others. Ruys[10] used it successfully with the addition of ascitic fluid. Later Cohn[11] modified it further, using veal infusion agar, ascitic fluid, and a filtered, veal broth cooked blood extract.

Media enriched with blood seem to have given to many investigators the most consistent results. Jenkins[12] found that citrated plasma agar alone gave satisfactory cultures. After testing many media and elaborating a special agar, Tulloch[13] obtained the best results with initial cultures when he added human or rabbit plasma plus one drop of whole human blood for each plate. Later Marshall[14] used a medium consisting of nutrient agar plus 5 to 10 per cent precipitated human red blood cells. Also Casper,[15] employing Bieling's method for the preparation of a pneumococcus medium, applied it to the gonococcus. He enriched an agar with horse blood, which was first hemolyzed by allowing a volume of blood to flow directly from the animal into two volumes of sterile distilled water. More recently some investigators obtained satisfactory initial gonococcus growths by the use of various chocolate agars (McLeod and co-workers,[16] 1934; Carpenter and co-workers, 1936[17] and 1938;[18] Reitzel and Kohl,[19] 1938).

From this brief survey of the literature, the important role which blood has played in the development of various gonococcus media becomes apparent. Media in which blood was used as enrichment seem to have been the simplest to prepare and the most reliable to use, since even dehydrated prepared agar, when enriched with it, gave good initial growth of the gonococcus (Reitzel and Kohl[19]).

However, the ideal medium for gonococcus isolations should be not only dependable and easy to prepare, but also one on which the characteristics of the gonococcus colony can be easily distinguished. It should, therefore, be colorless and transparent. In rendering blood media transparent and colorless, heating and filtering not only complicate the preparation of the medium,

but also greatly reduce the essential growth elements. As shown by the experiments of Torrey and Buckell,[20] the growth-stimulating substances of red blood cells are readily absorbed by materials ordinarily used to clarify media. On the other hand, Thjötta and Avery[21] found that even moderate heating impaired the growth accessory "V" factor essential for the cultivation of hemophilic bacilli. Wright[22] has also shown that overheating body fluids renders them inhibitory to the growth of the pneumococcus and other organisms.

It has been possible to prepare, in our laboratory, a simple and efficient medium consisting of veal infusion agar enriched with uncooked and unfiltered constituents of sterile citrated horse blood, namely, plasma and hemoglobin. The proportions for this plasma hemoglobin agar are 25 c.c. of veal infusion agar, 3 c.c. of sterile plasma, and 1 c.c. of clear hemoglobin in distilled water.

In preparing the veal infusion agar, the method described by Park and Williams[23] is followed.

The horse plasma is obtained by decanting with sterile precautions from whole citrated horse blood after the red blood cells have completely settled. Free hemoglobin may be obtained by bleeding the animal directly into an equal volume of sterile distilled water, or by mixing whole horse blood with an equal amount of water and keeping in the water bath at 45° C., with occasional shaking, until hemolysis takes place. The remaining red blood cells, from which the plasma has been decanted, may be diluted with 5 volumes of sterile distilled water which often causes hemolysis of the cells. If hemolysis does not then occur, the addition of a few drops of ether will lyse the red blood cells. There is good reason to believe that rabbit blood can be used almost as well as horse blood.

This plasma hemoglobin agar plate is transparent, practically colorless, since the reddish tinge fades in the incubator overnight, and it may be prepared the day before it is to be used. However, on freshly poured plates, the colonies are somewhat larger. We have, on at least two occasions, used plates which were five days old, and they have good growth of gonococci, though the colonies were small. Forty-eight hours after inoculation and incubation at 37° C., in an atmosphere containing ten to twelve volumes of carbon dioxide, the gonococcus colonies have the typical ground glass appearance and seem transparent when the plate is held up and light passes through them. Macroscopically the colonies usually appear glistening, round, raised, and with entire edge. Older colonies are usually irregular in shape and edge. There is, as a rule, a good growth of gonococci at the end of twenty-four hours, when grown under the above conditions. The viability of the gonococcus on this plate has been tested only up to one week. The transplants from week-old cultures all grew well.

The question whether cultures of all positive cases grow on a medium is a vital one. The only check the laboratory has on the efficacy of a medium is its comparison with the smear diagnosis and other known good media. The method of obtaining cultures from the patient, the transportation of the cultures to the laboratory, and the amount of inoculum planted on the plates are important considerations when evaluating primary gonococcus cultures on a medium.

Table I shows results obtained on freshly poured plasma hemoglobin plates compared with other good media and with smears.

TABLE I

NO. POSITIVE CASES		NO. POSITIVE ON PLASMA HEMOGLOBIN AGAR	NO. POSITIVE ON DIFCO AGAR*	NO. POSITIVE ON HORSE BLOOD WATER AGAR†	GRAM-STAINED SMEARS		
					POSITIVE	SUSPICIOUS	NEGATIVE
Vulvovaginitis	38	38	33	Not done	28	7	3
Vulvovaginitis	120	120	Not done	113	89	18	13
Male	44	44	42	44	42	0	2

*Difco proteose agar No. 3 and Difco dehydrated hemoglobin.

†Three parts of veal infusion agar plus 2 parts of horse blood water (equal volumes of horse blood and sterile distilled water).

Identifications on 2 per cent dextrose, 2 per cent maltose, and 2 per cent levulose agar plates, also on plain agar and 5 per cent washed blood agar, were carried out on all the strains included in Table I. Since the gonococcus colonies are more easily distinguished from similar colonies when grown on transparent and colorless media, fishings were usually made from the plasma hemoglobin plate before the oxydase test[24] was performed. This proved to be of importance because, when the dye was poured on a culture plate containing very few gonococcus colonies and a large growth of other flora, organisms were washed over the plate making pure cultures for identification impossible and often causing their loss. However, the oxydase test is clearly defined on the medium. The color changes are especially well seen when the plate being tested is placed on a white sheet of paper or held up to the light.

SUMMARY

An agar medium for primary gonococcus cultures is prepared by the use of veal infusion agar, horse plasma, and hemoglobin.

The advantages of the medium are:

1. It is efficient and easily prepared.
2. It is transparent and almost colorless.
3. The characteristics of the gonococcus colony on this medium are more easily distinguished from similar colonies.

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ISOLATION OF BACTERIA BY SURFACE-STROKE PLATING*

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IN THE course of some twenty-five years of teaching, the technique most essential to successful bacteriologic work, namely, the easy and practical isolation of pure cultures, is the very point least often stressed, or illustrated, in textbooks and laboratory manuals. This procedure is commonly passed over with a few words on "sun-burst" or parallel stroking of cultures on agar plates.

Furthermore, we have observed that the average student has more difficulty with successful colony isolation than with any other common bacteriologic routine. A method has been evolved in the attempt to overcome this deficiency. Its use in our hands, both personally and in classes, has yielded such constant and almost unvarying success that we feel that its use may well be advocated for trial by others who may have had similar difficulty. The procedure is illustrated in Fig. 1.

(1) In this method sterile agar plates are poured and well solidified. Using, preferably, a smooth platinum loop sealed in a light, hollow glass handle, a loopful of mixed culture (as from a Loeffler's throat culture) is carried to section A, and a small spot or smear is made at point marked "begin."

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(2) The loop is flamed to remove excess material, cooled, then stroked in parallel over approximately one-third of the plate area. The initial smear is touched only in the first three or four strokes.

(3) The plate is then rotated counterclockwise one-quarter turn, and the same area (A) is cross-stroked—again avoiding the initial spot of inoculation.

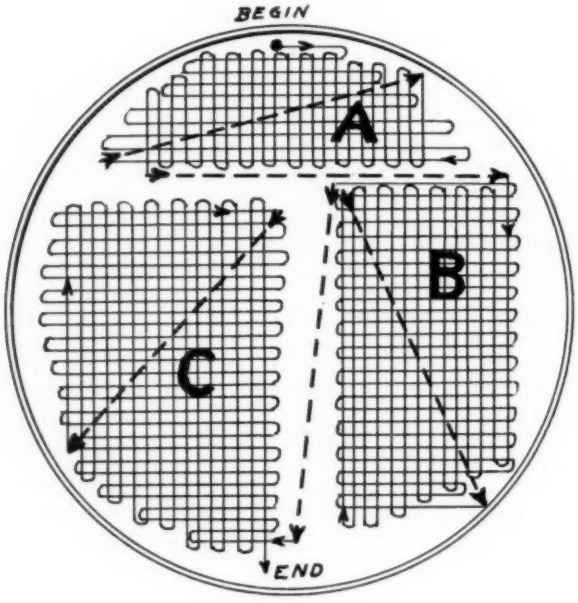

Fig. 1.

(4) Having cross-stroked section A, the loop is carried up to section B, and stroking is repeated—now avoiding contact with strokes in section A.

(5) Again the plate is rotated one-quarter turn, and section B is cross-stroked.

(6) The loop is then carried to section C and stroked—now avoiding contact with section B.

(7) The plate is turned the final one-quarter turn, and section C is cross-stroked.

The entire operation is accomplished in only some thirty seconds' time. Depending upon the numbers of bacteria placed on the original spot of inoculation, the plates invariably show optimum colony distribution in some section of the plates.

In 1934 we described a rotary inoculating table[1] which is still considered most useful for colony isolation. However, in the absence of this device, we feel that the method herein described will be found to be more uniformly successful than any of the procedures commonly used, either in private laboratory work, or in the instruction of beginning students.

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ANAEROBIC CULTIVATION AS A ROUTINE BACTERIOLOGIC PROCEDURE IN THE CLINICAL LABORATORY*

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IT WAS until recently a frequent experience in our clinical laboratory to observe microorganisms in smears of the original material which subsequently failed to develop in culture. Occasionally, it was necessary for the laboratory to submit an essentially negative report. In an effort to overcome the inadequacy of our cultural methods, a systematic attempt has been made to eliminate the incidence of negative cultures from specimens known to have contained bacteria.

There are at least four acceptable explanations for the failure of microorganisms to develop in primary culture. It is possible, for instance, that the specimen, when received, contained only dead bacteria, or that the amount of inoculum is inadequate. Nevertheless, it would seem more likely that the media are lacking in certain essential nutritive ingredients, or that the oxygen tension during incubation is too high. Preliminary trials with various culture media indicated that the nutritional factor played an important role but, at the same time, not the fundamental one. As a result, the comparative value of strictly anaerobic cultivation in the primary isolation of bacteria from clinical sources has been investigated.

Within the last few years many authors (Colebrook, 1930; Harris and Brown, 1929; McDonald and others, 1937; Schwarz and Dieckmann, 1927; Sears and Vinton, 1936) have called attention to the importance of anaerobic nonhemolytic streptococci. Topley and Wilson (1936) pointed out that anaerobic cultivation of primary agar plate cultures yields a higher incidence of hemolytic streptococci than do the corresponding aerobic plates. Smith and associates (1938) report a case of meningitis caused by a strictly anaerobic hemolytic streptococcus, and conclude that anaerobic cultivation of routine hospital material is important. Smith (1936) reported studies upon obligate anaerobic pneumococci. An interesting account of the sensitivity to oxygen of *Past. pestis* and other members of the Pasteurella genus was that of Schütze and Hassanein (1929). Furthermore, Dixon and Deuterman (1937) recently called attention to the possible pathogenic role of the members of the anaerobic genus, Bacteroides. It is evident, then, that, in addition to the anaerobes of war-wound infections, the importance of anaerobic, or reduced oxygen, cultivation in primary culture is amply indicated by the pertinent literature. Nevertheless, there appears to be little information regarding the prevalence of anaerobes in unselected routine cultures.

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The present report deals with the results obtained by comparing duplicate aerobic and anaerobic primary cultures on blood agar plates. A semisolid medium was also included in a small series. A total of approximately 2,500 consecutive routine specimens were examined by the method to be described. Of these 1,847 produced positive cultures.

Experimental Procedure.—Each specimen, as received, was streaked in an identical manner upon the surface of two blood agar plates. One Petri plate was labeled aerobic; the other was designated as the anaerobic culture and placed in a jar for anaerobic incubation. Some of the anaerobic plates were immediately subjected to anaerobiosis, but more often an interval of one to two hours intervened. The following morning the aerobic and anaerobic plates were examined together, and the resulting growths were compared. With a few exceptions all cultures were incubated for a minimum period of five days at 37° C., during which time they were examined daily for the appearance of new colonies. Further incubation depended upon the diagnosis submitted and the nature of the specimen.

No attempt was made to use selected specimens. Wherever specific culture methods were indicated, as in the isolation of *N. gonorrhoeae*, *Br. abortus*, or *C. diphtheriae*, the usual blood agar plates were inoculated in addition. All throat and feces cultures were included in the recorded series, but repeat specimens from the same patient were omitted. Since the use of agar plates has not been routine in the preparation of blood cultures, this type of specimen has not been considered. As a group, then, the specimens, upon which the present report is based, are representative of those received by any large hospital laboratory with the possible exception of bronchial secretions, which constituted 10 per cent of the cultures in our series.

Method of Producing Anaerobiosis.—To be practicable for the clinical laboratory an anaerobic procedure must be rapid, efficient, safe, and capable of accommodating a large number of cultures at one time. Furthermore, it is essential that such a method permit the use of agar plates, since only by the development of surface growth can a mixture of microorganisms be quickly and easily identified.

None of the many anaerobic methods known to us seems ideal. Toward that end one of us (E. H. S.) is devoting considerable effort, which will be made the subject of a future publication. In 1937 Weiss and Spaulding suggested a technique involving partial evacuation of a jar and the subsequent refilling with hydrogen catalyzed by palladium. Certain modifications in the technique and apparatus, as described below, have, we believe, resulted in a more practicable procedure. The data herein reported were obtained by this technique.

The essential system of two T tubes, a two-way stopcock (*A*) and a three-way stopcock (*B*) with downward outlet at end of the stopper, is illustrated in Fig. 1. A three-way stopcock, similar to A. H. Thomas Co., No. 9294, with 4 mm. bore is satisfactory. The glass apparatus may be suspended by metal clamps on ring stands or, preferably, placed in a grooved board and held in

place by metal strips. In place of an open mercury manometer, as originally described, a vacuum gauge has been substituted. Two types of gauges, especially designed for use with this apparatus, are available.* The first is subject to changes in atmospheric pressure, but is relatively inexpensive; the second is contained in a pressure-tight case and may be relied upon regardless of altitude or pressure. Both gauges have extra markings at 76, 640, and 710 mm. negative pressure. The gauge is attached to the glass system, as indicated in Fig. 1.

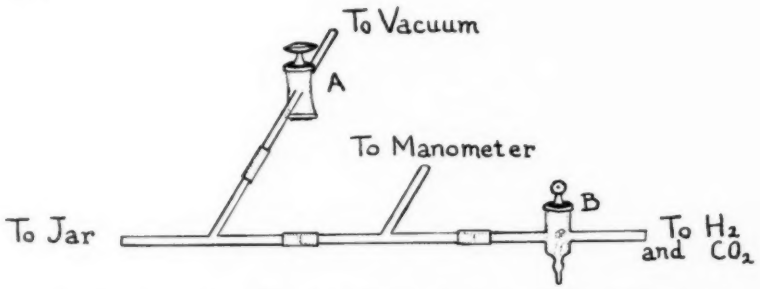

Fig. 1.—Drawing of the basic system of the anaerobic apparatus.

A Cenco-Hyvac, or similar oil pump, provides a rapid means of evacuation and is highly desirable where a large number of cultures must be handled. For the smaller laboratory, however, the slower but inexpensive water suction pump (at least 5⅜ inch length) will suffice. The pump is attached to the system by rubber pressure tubing, as indicated in the diagram.

Any jar of convenient shape and size may be used providing it can withstand the degree of vacuum required and is equipped with at least one stopcock. Several types of anaerobic jars are available, but they are usually expensive. The Hempel desiccator has proved satisfactory, but is not designed for convenient storage in the average incubator. We employ at present a chamber consisting of a glass cylinder (A. H. Thomas Co., No. 1085-A, flange at least ½ inch diameter) and a flat aluminum alloy cover with one stopcock extending horizontally from the side of the top. In a jar of this design the maximum of space is utilized. It will hold 14, 100 by 10 mm. plates and 25, 120 by 16 mm. culture tubes at one time. The inexpensive metal top is being manufactured at present by the Temple University machine shop.

The reduction of oxygen is accomplished by the catalytic power of palladium impregnated upon asbestos shreds. In this highly active form heat is not required, thereby minimizing the danger of explosion. It may be purchased from supply houses as 5.0 per cent palladiumized asbestos.

The procedure is carried out in the following manner: The inverted agar plates are placed in the jar, together with a flat porcelain dish (69 mm. diameter) containing 2 gm. of palladium. The catalyst should be located near the gas inlet. The criterion of satisfactory anaerobiosis is the reduction of methylene blue. The dye is added in a final concentration of 1:60,000 to meat extract broth, pH 7.6, containing 2.0 per cent dextrose. A small piece of thymol is

*United States Gauge Co., 522 Penfield Building, Philadelphia.

added as a preservative. It has been found convenient to keep the cotton-stoppered tube attached to the side of the jar with adhesive tape. The ground surface of the cover and of the cylinder are well smeared with a grease, such as Lubriseal (A. H. Thomas Co.) or a mixture of Cello-Seal and Cello-Grease (Fisher). The cover is put in place, and with pressure is rotated about the flange of the cylinder to produce a satisfactory seal. The jar is attached to the system by means of a rubber connection. With stopcock B closed, as in Fig. 1, and stopcock A open to the system, evacuation begins. This is continued until the manometer registers 710 mm. Turn stopcock A to disconnect the source of vacuum and to connect the system beyond stopcock B with carbon dioxide tank by means of rubber tubing. Without turning stopcock B rinse out the hose with the gas, shut off carbon dioxide tank, and open stopcock B by a 90 degree turn. The jar is now connected with the gas tank. Admit carbon dioxide until the gauge records a reading of almost zero (fifteen to thirty seconds). Close stopcock B and open A. When evacuation has again reached 710 mm., turn stopcock A and observe vacuum gauge for at least fifteen seconds for signs of a leak. If the pressure remains stationary, connect rubber hose to the hydrogen tank. It is more convenient to maintain a permanent connection with both gas tanks by inserting a Y tube in the rubber hose between stopcock B and the source of gas. Rinse out the tube through the outlet in stopcock B as before. Open stopcock B and admit the gas slowly; between thirty seconds and one minute should be consumed in refilling the jar. Turn the stopcock on the jar and disconnect from the system. The jar is now ready for the incubator. It may be desirable to use a clamp to hold the top in place. If, however, care is taken to keep the jar in a vertical position, this is not necessary.

The following morning the indicator should be colorless. To remove cultures, open stopcock, slide the cover off the cylinder, and remove the catalyst immediately. The top should never be put back on the jar unless the catalyst has been removed. An explosion may result. The catalyst should be heated over a Bunsen burner for five minutes to drive off moisture and then, upon cooling, is again ready for use.

By use of partial evacuation (710 mm. Hg) inversion of agar plates is possible. Because the technique is safe, rapid, and apparently efficient, its application to routine procedure appears justified. The apparatus above described has also been found ideal in producing an atmosphere of 10 per cent carbon dioxide as used in the isolation of Brucella, gonococci, and other carbon dioxidophilic bacteria.

Media Employed.—A total of 1,358 positive cultures were obtained upon a beef infusion blood agar prepared according to Wright (1933). The value of this medium, especially for the isolation of fastidious pathogens, is believed to be due in part to the reduction of certain oxidized constituents in the peptone. A second series of 489 positive cultures were examined after isolation upon a nutrient blood agar containing 1.0 per cent Bactopeptone, 0.3 per cent Difco meat extract, and 0.5 per cent sodium chloride to which was added 6.0 per cent human citrated blood. It was hoped that, by comparing media of

two different growth abilities, the relationship between the nutritive quality of the medium and the oxygen requirements of these bacteria might be indicated. All media were used within twenty-four hours after preparation.

Experimental Results.—The comparative aerobic and anaerobic growths upon both types of media are presented in Table I. No consideration is given to the various species present.

TABLE I

COMPARISON OF THE NUMBER OF POSITIVE CULTURES ON AEROBIC AND ANAEROBIC BLOOD AGAR

AEROBIC PLATE	ANAEROBIC PLATE	MEAT EXTRACT BLOOD AGAR		BEEF INFUSION BLOOD AGAR	
		NO. OF SPECIMENS	PERCENTAGE	NO. OF SPECIMENS	PERCENTAGE
+	+	373	76.3	1223	90.0
+	0	24	4.9	16	1.2
0	+	92	18.8	119	8.8
Total		489	100.0	1358	100.0

Since Wright's medium contains an infusion base and is prepared in such a manner as to contain reduced substances, one might expect, in comparison with the meat extract agar, to find a less striking difference between the aerobic and anaerobic plates. Table I supports this expectation. On the meat extract medium, 18.8 per cent of the specimens produced positive cultures only by anaerobic cultivation, whereas the corresponding figure on infusion agar was 8.8 per cent. Since the series included a large proportion of feces specimens in which negative cultures never occurred, this figure assumes added significance. Furthermore, it is our belief that the figure of 8.8 per cent represents those specimens which, prior to the inauguration of anaerobic cultivation, remained sterile in spite of positive microscopic evidence. On the other hand, a surprisingly small number of specimens produced aerobic growth exclusively.

TABLE II

ANALYSIS OF SPECIMENS SHOWING ONLY ANAEROBIC GROWTH

TYPE OF ORGANISM	NUMBER OF ISOLATIONS	
	MEAT EXTRACT BLOOD AGAR	BEEF INFUSION BLOOD AGAR
Hemolytic streptococcus	40	50
Nonhemolytic streptococcus	37	30
Pneumococcus	13	16
Gram-negative cocci	21	15
Bacteroides	7	14
Diphtheroids	8	11
H. influenzae	0	3
Miscellaneous	11	10

Analysis of Specimens Containing Only Anaerobes.—If the anaerobes isolated from aerobic-negative specimens consist only of contaminants and nonpathogenic types, there would be considerable doubt as to their routine value in the clinical laboratory. An examination of Table II, however, reveals that the most common organism recovered under these conditions was the hemolytic streptococcus. In order of decreasing frequency were the nonhemolytic strep-

tococci, a heterogeneous group of gram-negative cocci, pneumococci, and bacteroides. It is of interest that the Clostridium group was only occasionally encountered among these specimens.

Anaerobic Hemolytic Streptococci.—Because of the importance of hemolytic streptococci in human infections, the incidence of anaerobic hemolytic streptococci in the entire series of specimens has been tabulated. The results appear in Table III.

TABLE III

HEMOLYTIC STREPTOCOCCI

Frequency of Anaerobic Isolations

AEROBIC PLATE	ANAEROBIC PLATE	MEAT EXTRACT BLOOD AGAR		BEEF INFUSION BLOOD AGAR	
		NUMBER OF SPECIMENS	PERCENTAGE	NUMBER OF SPECIMENS	PERCENTAGE
+	+	71	33.2	270	56.3
+	0	5	2.3	4	0.8
0	+	138	64.5	206	42.9
Total		214	100.0	480	100.0

It will be seen by examining Table III that more than 40 per cent of the hemolytic streptococci isolated on infusion blood agar failed to appear on the aerobic plates. The anaerobic strains originated from many different pathologic conditions, and in no way suggested a predilection for any one part of the body or disease process. No attempt has been made to determine the serologic grouping of these strains.

The high percentage of anaerobic hemolytic streptococci occurring in the meat extract series may be accounted for by the use of a medium of low nutritional value. Since almost 65 per cent of the isolations were recovered only on the anaerobic plate, it would appear that anaerobiosis may, to some extent, compensate for a poor culture medium.

An observation of considerable practical importance has received no consideration in compiling these data. It has been customary to observe the development of hemolytic streptococci, and sometimes pneumococci, within twenty-four hours on the anaerobic plates, whereas the corresponding aerobic plate might require two or three days before revealing similar colonies. Since growth occurred under both conditions, it was tabulated accordingly, but the practical significance of the more rapid anaerobic growth is obvious. Not only did many of the anaerobic cultures develop earlier, but, in addition, they produced more luxuriant colonies and larger areas of hemolysis.

Anaerobic Pneumococci.—The isolation of pneumococci, which at the same time failed to appear on the primary aerobic plate, was not an uncommon occurrence. In many instances, but not all, these organisms were identified biochemically and typed by the Neufeld reaction. The "greening" of blood agar and hemolysis tend to be suppressed, but to a varying degree reappear upon exposure to air. An outstanding instance in which an anaerobic culture resulted in the early institution of therapy was a type VII pneumococcus meningitis. In spite of negative microscopic evidence of bacteria in the original material, the anaerobic plate after eighteen hours' incubation revealed several

colonies which were immediately typed. The aerobic plate, as well as those from succeeding specimens of spinal fluid, remained uniformly sterile. The data relating to the isolation of pneumococci on both types of media are presented in Table IV.

TABLE IV

PNEUMOCOCCI

Comparison of Duplicate Aerobic and Anaerobic Blood Agar Plates

AEROBIC PLATE	ANAEROBIC PLATE	MEAT EXTRACT BLOOD AGAR		BEEF INFUSION BLOOD AGAR	
		NUMBER OF SPECIMENS	PERCENTAGE	NUMBER OF SPECIMENS	PERCENTAGE
+	+	53	57.6	227	82.9
+	0	16	17.4	22	8.0
0	+	23	25.0	25	9.1
Total		92	100.0	274	100.0

Other Anaerobic Types.—In the infusion series were 213 isolations of various nonhemolytic streptococci. Of these 152 appeared on both plates, but 59 were anaerobic only. No attempt to classify these strains according to Prévot (1925) has been made. It is interesting also that of 28 cultures of *H. influenzae* recovered, 13 appeared only with reduced oxygen tension. Of the 14 isolations of Hemophilus which developed on both plates, it was not uncommon to observe growth appearing aerobically only in the vicinity of staphylococcus colonies (satellitism), whereas a large number of uniformly distributed colonies occurred on the corresponding anaerobic plate. This observation, although not seen in all isolations, was made frequently enough to raise some doubt as to whether satellitism depends entirely upon the elaboration of V factor. Since growth of certain strains resulting from the same inoculum may show satellitism aerobically and yet develop independently of staphylococci in the absence of oxygen, it is possible that a growth-stimulating substance, or substances, available only through the agency of staphylococci under aerobic conditions, is present in an assimilable form in the absence of oxygen.

Viridans streptococci seem less prone to develop anaerobically than do other types of streptococci or pneumococci. When growth does occur, "greening" is frequently suppressed so that the organism appears as a nonhemolytic streptococcus. Re-exposure to air usually results in subsequent discoloration.

Semisolid Medium.—A small proportion of the specimens were at the same time inoculated to a medium of the same composition (infusion), but to which had been added 0.3 per cent agar. The results were, as might be expected, intermediate between those of the aerobic and anaerobic plates. Nevertheless, in no instance in which the semisolid medium was positive, did the anaerobic plate fail to show growth. No medium especially designed for anaerobes, such as brain or cooked meat broth, was used. Any fluid or semisolid medium would have the same disadvantage that impelled us to restrict our further studies to the use of agar. A medium, such as Rosenow's brain broth, would undoubtedly permit the development of a large proportion of our "anaerobic" isolations. On the other hand, the separation and identification of a mixture

of bacteria would still require subcultures to aerobic, if not anaerobic, blood agar. The additional time and effort can be avoided by the direct inoculation of duplicate agar plates. Those laboratories, however, in which suitable anaerobic equipment is not available, might well use semisolid or meat media with subsequent subculture to aerobic blood agar. It should be pointed out that two disadvantages are inherent in this procedure. During incubation of a mixed culture in fluid media the more delicate pathogens may be completely overgrown or inhibited and, because of the necessary reinoculation to agar, additional time is required.

The addition of a reducing agent (0.03 per cent cysteine hydrochloride) to the medium was attempted in a small series of specimens with the thought that the presence of the cysteine would, perhaps, permit the growth of the anaerobic types of streptococci. This medium was quickly discontinued, however, because of the rapid and extensive production of "greening" about many different types of colonies, due, no doubt, to the formation of sulfhemoglobin.

Permanence of Anaerobic Requirements of Hemolytic Streptococci and Pneumococci.—The hemolytic streptococci and pneumococci, with which we are here largely concerned, became, with few exceptions, quickly adapted to aerobic environment. In the large majority of instances the first transplant from anaerobic to aerobic blood agar resulted in varying amounts of growth. No effort was made to determine the exact proportion of permanently anaerobic hemolytic streptococci or pneumococci. Nevertheless, permanently anaerobic strains have been isolated without difficulty for experimental purposes. The occurrence of this type, however, would seem to be relatively uncommon.

DISCUSSION

The significant number of hemolytic streptococci and pneumococci isolated only by the use of anaerobic incubation would alone seem to justify the adoption of routine anaerobiosis in the clinical laboratory. In addition, the isolation of anaerobic nonhemolytic streptococci, actinomyces, *H. influenzae*, Bacteroides and Clostridia has suggested etiologic relationship to a number of unusual, and hitherto undiagnosed, cases. Indeed, it is our opinion that a satisfactory bacteriologic examination is possible only if an anaerobic culture is included. Soule (1932), in discussing the stimulus to the investigation of anaerobes resulting from the World War, says ". . . . . a bacteriologic study of unknown material was not complete until the question of the presence of anaerobes was satisfactorily disposed of. Nevertheless, it is a matter of common occurrence to find that the knowledge reawakened by the war studies has lapsed and today the average complete bacteriologic examination of suspected materials consists of the isolation and attempted identification of the aerobic flora." We wholly agree with this opinion in so far as it applies to clinical bacteriology laboratories. It is our hope, therefore, that data herein presented may serve to emphasize the frequency with which anaerobic members of well-recognized aerobic, or facultative anaerobic, species are encountered in primary isolation.

The fact that such a large proportion of hemolytic streptococci require, or are stimulated by, lowered oxygen tension suggests that many strains may be essentially anaerobic. In support of this hypothesis is the continual observation that most laboratory strains develop more readily under anaerobiosis. If these organisms are not fundamentally anaerobic, one must, then, necessarily assume that the inclination toward anaerobic behavior is the result of adaptation in the human body to an environment in which the oxygen tension is low. Evans and Hartridge (1936) have written, "It has long been known that dyes such as methylene blue can often be reduced by most tissues." Thus, it would seem that normal tissue may have an E_h of $\pm$ 0.0 millivolts, and consequently one may readily imagine that debilitated and traumatized tissue, especially with a diminution in the blood supply, may provide even more anaerobic conditions. By the process of adaptation, the mechanism of anaerobic respiration may assume a prominent, and more or less permanent, character which must inevitably be reflected upon inoculation to culture media. Under these conditions the surface of anaerobic blood agar perhaps simulates conditions within the body more closely than does the corresponding aerobic plate. The fact that certain strains persist in their anaerobic requirements leads one to the supposition that the period of adaptation was in those instances of considerable duration. There is no evidence, however, to suggest that the anaerobic strains are more virulent than the aerobic types.

By far the majority of anaerobic hemolytic streptococci and pneumococci developed the capacity for growing aerobically on the first subculture. These we have termed "temporarily anaerobic." Prévot[6] has given the name "anaerobes of predilection" to those organisms behaving less rapidly in the same general way. No attempt has been made, however, to determine their serologic grouping. Of practical significance is the fact that a medium permitting the development of anaerobes, such as deep tubes of brain broth, will unquestionably recover most of the temporary anaerobes. If overgrowth by a less fastidious organism and bacterial antagonism can be overcome, subculture to aerobic blood agar will perhaps be a useful procedure in the small laboratory.

A further comparative advantage of the anaerobic blood plate is the definite tendency of anaerobic cultivation to inhibit the common aerobic contaminants. It is not infrequent, for instance, to observe a tracheal secretion culture showing nonhemolytic staphylococci and *N. catarrhalis* aerobically, but a pure culture of pneumococci or streptococci on the anaerobic plate.

CONCLUSIONS

1. Approximately 2,500 routine, clinical specimens have been cultured in duplicate upon aerobic and anaerobic blood agar plates.

2. The total number of specimens was divided into two series using, for the first, beef infusion, and for the second, meat extract base.

3. A percentage of 8.8 of the specimens of the meat infusion group was positive on the anaerobic plate but remained sterile aerobically. By the use of meat extract medium, an incidence of 18.8 per cent was obtained.

4. The most common organism appearing on primary isolation only after anaerobic cultivation was the hemolytic streptococcus. Anaerobic pneumo-

cocci were also frequently isolated. A possible explanation for the occurrence of these anaerobic types is discussed.

5. By far the great majority of these organisms quickly regained their ability to grow aerobically. A method is suggested for utilizing this adaptability.

6. A practical procedure for routine anaerobiosis in the clinical laboratory is recommended.

7. The occurrence of negative cultures from specimens showing a positive smear has been almost entirely eliminated.

8. Bacteriologic studies in the clinical laboratory are incomplete without anaerobic culture.

We wish to express our indebtedness to Dr. Ezra Casman, of the Abington Memorial Hospital, for his suggestions and helpful criticisms.

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A SIMPLE METHOD FOR DETERMINING BACTERIAL REDUCTION OF NITRATES*

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THE routine method of testing bacterial cultures for nitrate reduction consists in inoculating broth containing potassium nitrate, and after incubation adding a reagent which produces a color in the presence of the nitrite radical. An alternative method consists in inoculating agar containing potassium nitrate and after incubation observing the culture for the presence of gas. A large amount of effort has been expended in devising a culture medium which would give consistent results in testing for nitrate reduction to nitrites.

Stickland[1] reported that the untreated cells of *E. coli* reduce nitrate to nitrite with great rapidity. We have made the same observation, and it occurred to us to extend the work to include different species of bacteria, and to compare the method to be described with the routine method.

METHOD

The basis of the method consists in adding a solution of potassium nitrate to a suspension of the living bacterial cells to be tested. From fifteen minutes to one hour later the mixture can be tested for the presence of nitrites.

In our work we have used either twenty-four-hour broth cultures or some of the growth from an agar slant emulsified in saline. To 1 c.c. of culture, 0.2 c.c. of 1 per cent potassium nitrate is added. The mixture is shaken and allowed to stand for about one-half hour when it is tested for the presence of nitrite. If desired, quantitative determinations can be made at intervals and the rate of reduction determined.

We have tested 24 different species of bacteria using the routine method and the modified method, and we have found that there is complete agreement between the two. The following organisms reduced nitrates to nitrites by both methods: *Staph. albus, Staph. aureus, B. subtilis, B. anthracis, E. coli, A. cloacae, E. typhosa, S. paratyphi, S. schottmülleri, S. enteritidis, S. pullorum, S. gallinarum, Brucella abortus, Shigella paradysenteriae* (Flexner), *Shigella paradysenteriae* (Sonne), and *C. hofmannii.* The following organisms failed to reduce nitrates by either method: *Shigella dysenteriae, Alcaligenes faecalis, Vibrio metchnikovii, Strep. faecalis, Strep. hemolyticus, Strep. viridans, C. diphtheriae,* and *C. xerosis.*

DISCUSSION

The simple method described has several advantages. A special medium is not required. Organisms difficult to grow in liquid media may be grown on

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enriched solid media, and the emulsified growth used in the test. Since the rate of reduction is increased if the concentration of organisms is increased, bacteria which grow sparsely may be concentrated by centrifugation and tested. With this method organisms which break down the nitrate radical to nitrite and then utilize the nitrite, and which would be recorded as negative by the routine method, will very likely be positive with the modified method. Old cultures in which most of the bacteria are dead will not reduce the nitrate. Media prepared with filtered material should not be used in growing bacteria to be tested, as Berkefeld and Seitz filters contain nitrite.

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A RAPID STAINING METHOD FOR OPSONOCYTOPHAGOCYTIC INDICES*

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WHILE working on a problem in which phagocytosis was concerned, we[1] developed a stain which we found very efficient. In view of the great amount of work now being done on the opsonocytophagocytic index in Brucella infections, it is likely that this method may find a field of usefulness.

The stain is made by adding 0.15 gm. methyl green and 0.5 gm. pyronine to 15 c.c. of 95 per cent alcohol. It is best mixed in a mortar. Eighty-five cubic centimeters of 3 per cent carbolic water is added slowly until the stain is completely dissolved. The completed mixture should stand at least a week with occasional shaking before it is used. Smears are made in the usual manner, dried in air, and flooded with the stain. They are allowed to stain four minutes, washed in tap water, dried in air, and examined. The cytoplasm of the white cells stains a faint pink, the nucleus a light red, and the organisms a deep red. The granules in the cytoplasm do not stain, and it is this feature which so much facilitates the bacterial count. When the usual blood stains are used, there is always some confusion between bacteria and cytoplasmic granules.

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A SLIDE FLOCCULATION TEST FOR THE DIAGNOSIS OF SYPHILIS*

Preliminary Report

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THE serologic diagnosis of syphilis is based upon two types of reaction, viz., the complement fixation phenomenon discovered by Bordet and Gengou[1] and adapted by Wassermann, Neisser, and Bruck[2] for the diagnosis of syphilis; and the precipitin reaction observed by Porges and Myer[3] and adapted for the diagnosis of syphilis by Michaelis.[4] Most likely both reactions are similar in nature in that they both depend upon the formation of a precipitate by the union of the "reagin" present in the serum globulin, with the lipoidal colloids of the antigen, and the subsequent adsorption of the complement by this precipitate. This theory is supported by the fact that examination of Wassermann reaction mixtures of syphilitic serum and antigen by the ultramicroscope shows the presence of precipitates even though these precipitates are invisible macroscopically.[5] The difference between the complement fixation and precipitin reactions seems to lie in the physical conditions which determine the size of the particles in the precipitates.

A great deal of attention has been paid to the development of suitable precipitin tests because these tests are speedier, easy to perform, and require fewer reagents. The most important advance in this field was made by Kahn[6] who produced a visible precipitate in syphilitic serum by using highly concentrated and sensitive antigen suspensions. To further enhance the visibility of the Kahn precipitate the centrifugation and resuspension of the precipitate in saline were suggested.[7] The foundation of the Kahn test is based upon four factors: namely, optimum concentration of ingredients, great instability of the antigen-saline suspension, quantitative relationship between antigen suspension and serum, and agitation of the ingredients. On the basis of these principles precipitin tests were devised by Kline,[8] Eagle,[9] Laughlen,[10] and others.

The slide flocculation test described in this paper has the following advantages: the antigen is stable and highly sensitive to syphilitic serum, the quantitative relationship between antigen and saline, and between antigen-saline suspension and serum, need not follow the strictest measurements but may be varied within certain broad limits without affecting materially the efficacy of the test. Other advantages are speed, easy manipulation, and good visibility.

Preparation of the Antigen.—Fresh beef heart is used. Because of qualitative and quantitative variations in the lipoidal content of hearts obtained from different animals, at least six hearts should be used. After removal of fat

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and other adventitious matter, the muscular tissue of the hearts is cut up into small pieces and passed through a meat grinder a few times until a very fine paste is obtained. The paste is spread in very thin layers over cheese cloth stretched tightly on wooden frames and dried continuously for two days at room temperature by means of an air blast. The dry tissue is broken up into small pieces and ground to a very fine powder. One hundred grams of the dry powder are shaken continuously in a liter flask with 400 c.c. of pure anesthesia ether for ten minutes and filtered through a fat-free filter paper. The powder on the filter paper is pressed lightly with a spatula until no more ether passes through the funnel. The moist powder is returned to the flask and again extracted with 400 c.c. of ether, following the same procedure as in the first extraction. This is repeated once more, making three extractions in all, with a total volume of 1,200 c.c. of ether. After the third extraction the moist powder is spread on a glass plate or on a clean paper and dried at room temperature until the odor of ether is completely gone. A weighed quantity of the ether-extracted powder is introduced into a dry flask, and absolute alcohol is added in the proportion of 5 c.c. of alcohol to 1 gm. of powder. The flask is covered tightly with a cork wrapped in tin foil and allowed to stand in a dark place at room temperature for four days. It is shaken daily for five minutes. At the end of the fourth day it is filtered through a double layer of fat-free filter paper and is cholesterinized with 6 mg. of pure cholesterol per cubic centimeter of extract. When the cholesterol is completely dissolved, it is allowed to stand at room temperature for twenty-four hours and filtered. About one-tenth volume of the alcoholic extract is transferred to a small flask and shaken vigorously with small amounts of dimethylamidoazobenzol until a saturated solution is obtained as evidenced by an excess of the dimethylamidoazobenzol. This is filtered through a double layer of fat-free filter paper into a small graduated cylinder, and three volumes of the alcoholic extract are added to the cylinder and mixed well. The rest of the alcoholic extract is saturated with sudan III dye and filtered. The two solutions are now mixed in the proportions of 85 volumes of the sudan III extract to 15 volumes of the dimethylamidoazobenzol and shaken well. The antigen is now ready for use. Antigens thus prepared eighteen months ago were found to contain their full potency.

Performance of the Test.—An antigen-saline suspension is prepared by placing a measured volume of antigen in a wide test tube, about 15 mm. outside diameter, and adding rapidly to it two volumes of 0.9 per cent sodium chloride solution. The mouth of the test tube is closed with a clean cork covered with tin foil, and the mixture is shaken rapidly with an up-and-down motion for one minute. The suspension may be used immediately and will not lose its potency for at least twenty-four hours. The serum is inactivated in the usual manner at 56° C. for half an hour. Two drops of inactivated serum (approximately 0.08 c.c.) are placed on a clean smooth glass slide, and one drop (approximately 0.03 c.c.) of the antigen suspension is dropped directly on the serum from a distance of about 1 cm. The serum and antigen are mixed together well by means of a thin glass rod, thereby spreading out the mixture

to form a circle about 15 mm. in diameter. The slide is now rotated with a gyratory motion for three minutes. In strongly positive sera large red floccules appear within a few seconds; after a minute or two of rotation, the floccules accumulate at the edges of the circle, leaving a clear colorless medium in the center. In weakly positive sera flocculation appears more slowly, in from one to three minutes, depending upon the amount of syphilitic reagin in the serum, and the floccules are of a smaller size and fewer in number. With a little experience, one soon learns to judge the strength of a

Figs. 1, 2, and 3.—*Gross appearance* (as seen with the naked eye). *1*, Negative; *2* and *3*, Positive.

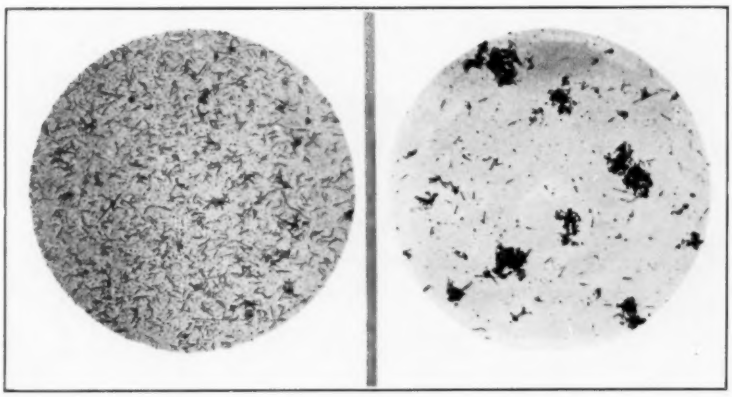

Fig. 4. Fig. 5.

Figs. 4 and 5.—*Microscopic appearance* (under low power ×320). *4*, Negative; *5*, Positive.

positive reaction by the number and size of the floccules and by the speed with which they appear. Nonsyphilitic serum shows no flocculation whatsoever, and the edges of the circle remain smooth. When examined under the low-power lens of the microscope, negative serum shows a large number of red, slender needle-shaped crystals, somewhat thickened in the center and pointed at both ends. These crystals are evenly distributed throughout the microscopic field. Syphilitic serum shows large clumps of crystals surrounded by clear spaces.

Close to 10,000 tests were performed by this procedure during the last year and a half and compared with the Wassermann and Kahn tests. Good agreements were obtained with both the Kahn and the Wassermann tests. Particularly good agreements were obtained with 2,000 Wassermann tests performed with a highly specific cholesterinized antigen obtained through the courtesy of the New York State Health Department. The Wassermann tests performed with that antigen checked well with the clinical histories.

Because of these encouraging results, this simple and rapid method is recommended to the syphilologist in the hope that it may prove a valuable aid to him in the diagnosis and treatment of syphilis.

I wish to express my gratitude to Dr. J. C. Ehrlich, Director of Laboratories, Lebanon Hospital, for his invaluable help and encouragement in this work.

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AN IMPROVED TEST TUBE RACK*

Robert F. Ervin, M.S., Notre Dame, Ind.

THERE is need for a test tube rack which will hold test tubes so firmly that they will not rattle nor slip out. The test tube rack described here was designed to eliminate some of the objectionable features found in most racks. Tubes are usually supported in holes punched in plates of metal or wood. Application of this principle makes it impossible to design a proper rack which will hold a variety of tubes tightly. Moreover, most metal racks and basket arrangements are easily damaged by bending and corrosion, and are difficult to repair. More than this, if a rack is to be used for tubes of varying sizes, only the tubes of uniform diameter will be held securely; all other tubes, unless supported at several points, will rattle and slip out.

It is felt that these disadvantages have been overcome in the rack illustrated in Fig. 1. The design consists primarily of a zigzag bar, supported at each end by uprights from a flat base. The entire construction is cast in one piece from white metal alloy, and is cadmium plated. A phosphor bronze coil spring is run down each side of the rack through holes in the apex of each V in the zigzag bar. These springs are fastened securely at each end by metal tapping screws. The standard model rack is 12¾ inches long, 2 inches wide at the base, and 2½ inches high. It will accommodate tubes of ⅜ inch to ¾ inch outside diameter, and has a capacity of 20 tubes.

*From the Laboratories of Bacteriology, University of Notre Dame, Notre Dame.
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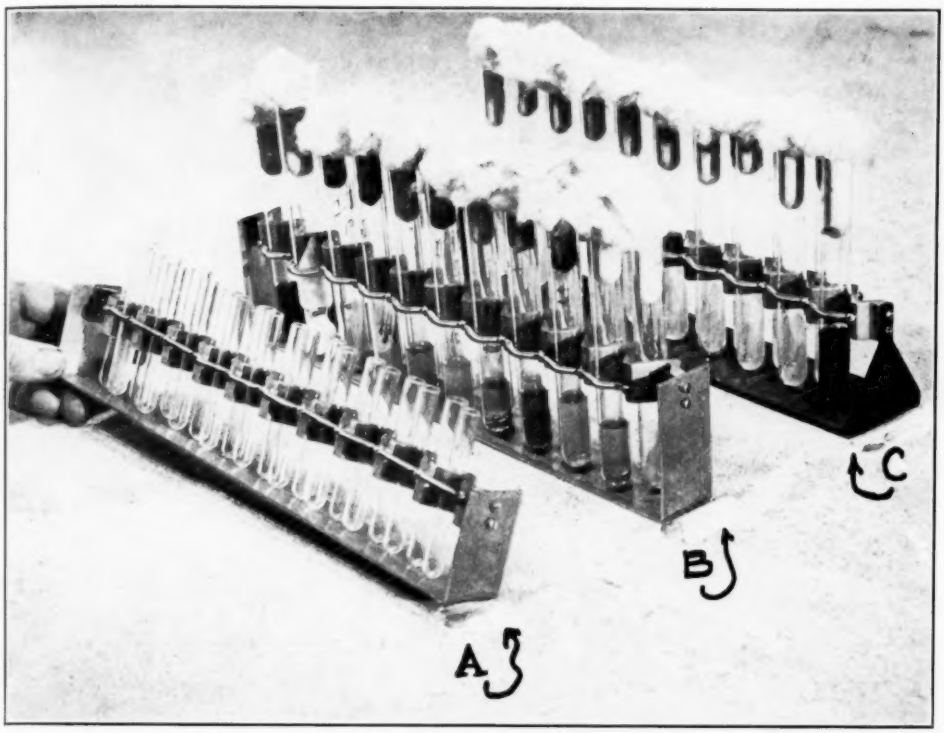

Fig. 1.—*A*. Clinical model (for ¼ inch to ⅜ inch O.D. tubes). *B*. Clinical model (for ⅜ inch to ¾ inch O.D. tubes). *C*. Standard model (for ⅜ inch to ¾ inch O.D. tubes).

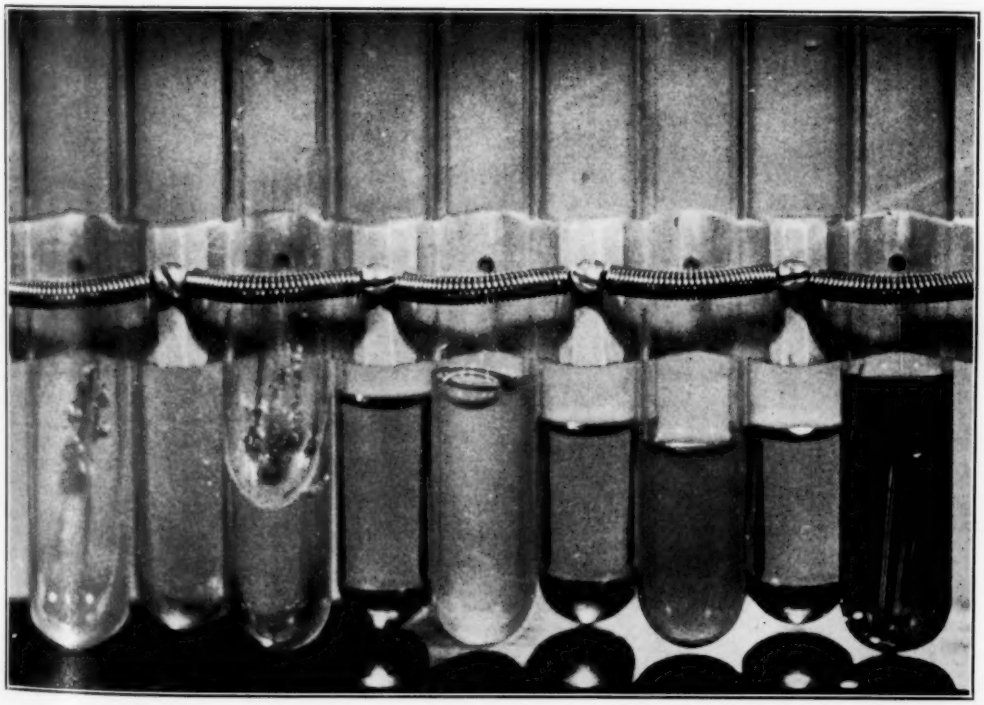

Fig. 2.—All tubes are visible from one position.

When in use, the tubes are held firmly in the V-shaped slots by the coil spring and rest against the base of the rack. They are easily slipped in and out of the slots, but are held so tightly in place that the rack may be turned upside down without causing the tubes to fall out.

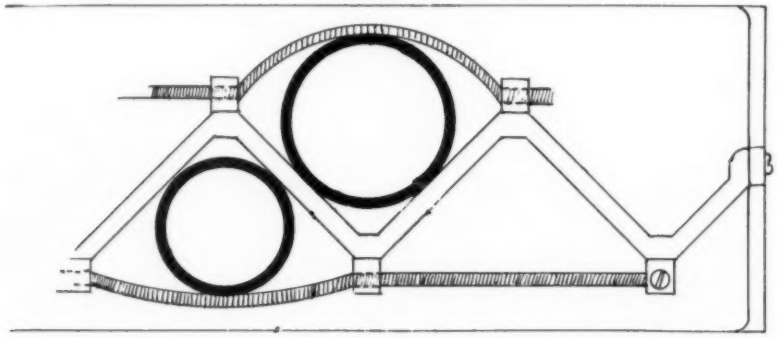

Fig. 3.—Showing three point support and continuous spring detail of the test tube rack.

The simple, sturdy construction makes cleaning easy and provides a rack which will endure hard use in a laboratory for years. The use of the zigzag bar makes all tubes visible from one position without removing any of them from the rack. This is a decided advantage when it is necessary to compare reaction-tubes with controls. The manner of holding the tubes allows the rack to be shaken in a shaker or by hand without rattle or danger of slipping. The design of the rack as well as the space-saving way of holding the tubes guarantees its efficiency for use in water baths, incubators, ice chests, etc.

In summary, a new test tube rack has been designed, and its efficiency has been proved in the laboratory. Its advantages are simplicity of design, ease of use, sturdiness of construction, economy of space, and rigidity as a support for test tubes.

This test tube rack is manufactured and distributed by Rayno Research, 3806 N. Ashland Ave., Chicago, Ill. After the design of Professor James A. Reyniers.

AN ALL-GLASS ADJUSTABLE MOUSE CAGE*

THOMAS W. RAY, PH.D., MILWAUKEE, WIS.

IT BECAME necessary to develop an all-glass adjustable mouse cage of sufficient size to hold a considerable number of animals. This type of cage is convenient to use and embodies the following special features: (1) ample ventilation, (2) glass wheels for exercising, (3) an attached drinking fountain, (4) ease in disassembling completely or in sections for cleaning, (5) convertible into one, two, three, or four units.

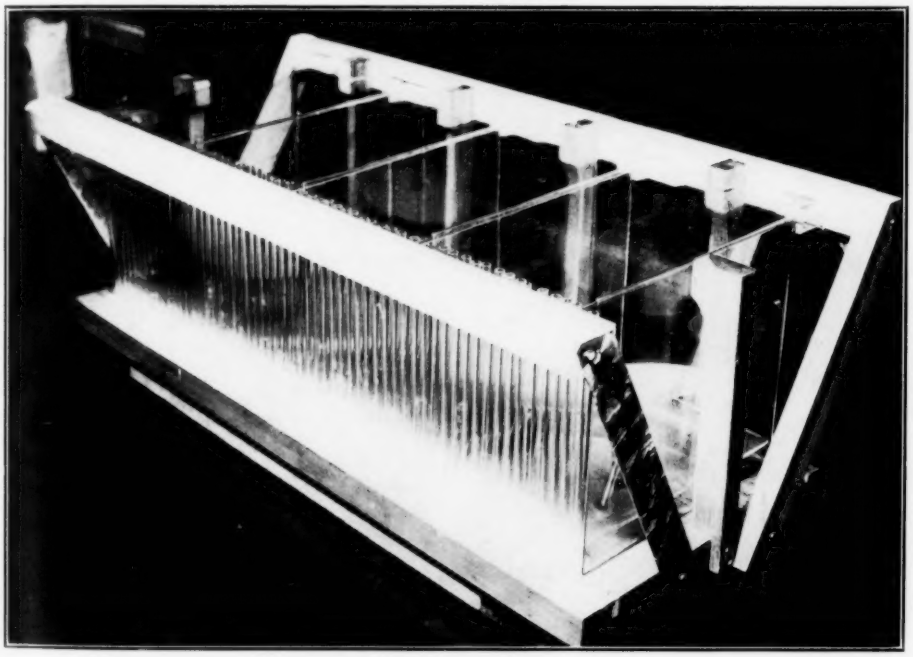

Fig. 1.

In a board 1½ inches thick, 12 inches wide, and 28 inches long, a groove ¼ inch wide and ½ inch deep was made parallel with, and 1 inch from, the back, the full length of the board. At right angles to this groove, five similar ones were made, each 6 inches apart and 9 inches long, dividing the board into four 9 by 6 inch sections.

Plates of double strength glass, 9 inches by 9 inches, placed on edge in the grooves, form the back, end walls, and the partitions, while glass tubes

*From the Department of Biochemistry, Marquette University Medical School, Milwaukee.
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8 mm. in diameter cemented in holes bored 4 mm. apart through the board, and parallel with the edge, serve as the front and allow for ventilation. Suitable braces support the back and ends from the outside, while the middle partitions keep the back plates from tilting forward. The four sections are covered by plates, 6 inches by 12 inches, whose edges fit into grooves in the back braces. These top plates slope slightly toward the front, resting on the ends of the glass tubes and fitting between the end walls and the middle partitions, holding them erect. The front edges of the tops slip into a groove in a narrow board and are held down and back by latches securing the ends of the board to the cage.

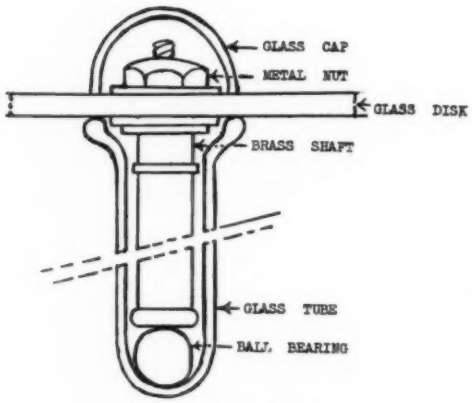

Fig. 2.—Exercise wheel.

Fig. 3.—Drinking fountain.

In the corners of each of the four sections holes, ¼ inch in diameter and ½ inch deep, are made in the board. Glass tubes placed in these holes and extending 1 inch above the board serve as supports for the glass floors.

A glass disc, 5½ inches in diameter, frosted on the top side, to which is fitted a brass shaft, provides an exercise wheel. The lower end of the shaft rests on a steel ball in the bottom of a glass tube. The metal nut above the disk, and securing the disk to the shaft, is covered with a glass cap, while the shaft is completely protected by the glass tube. The wheel is held in position for use by inserting the shaft, covered by the glass tube, into a hole bored at a slight angle through the glass floor and into the board below.

Any one, or all, of the plates dividing the cage into sections may be removed, and plates, which extend only 2 inches above the glass floor, may be substituted, thus making it possible to convert the cage into one, two, three, or four units as desired.

The mice may be confined in any one small section while the other parts of the cage are being cleaned. A glass drinking tube is held erect and secure by inserting it into a glass cylinder and slipping the cylinder over two of the glass tubes forming the front of the cage. A short cylinder is first passed over the two tubes holding the mouth of the drinking fountain the desired height above the floor.

A NEW SAHLI TYPE HEMOGLOBINOMETER*

RUSSELL L. HADEN, M.D., CLEVELAND, OHIO

GOWERS first suggested in 1878[1] the quantitative estimation of hemoglobin by diluting a fixed amount of blood in a graduated tube until the color matched that of a standard in a tube of equal diameter. The blood was diluted with distilled water and compared with a solution of picrocarmine in glycerin. A few years later Sahli[2] modified this method by converting the blood into acid hematin by mixing with N/10 hydrochloric acid and comparing it with a solution of acid hematin. Haldane[3] still later used a carbon monoxide-hemoglobin standard and converted the hemoglobin of the unknown blood into this compound by bubbling illuminating gas through it.

The Sahli method of hemoglobin determination has long been a favorite one. The apparatus is inexpensive, and the brown color of hematin is easily matched. It is necessary that the bore of the standard and the graduated tube be exactly the same, the pipette must be accurate, and the standard must be correct. The liquid standards of acid hematin were soon found unsatisfactory because of fading and other difficulties and have been replaced by glass standards to match acid hematin. The round tube has also been replaced by a square one which is made over a metal core and so has a uniform inner diameter.

Even after the square tube and an accurate glass standard which matches acid hematin closely are provided, one difficulty with the Sahli method remains. The glass standard is the same throughout its length. The blood is gradually diluted until it matches the standard. It is very difficult to tell when this match is correct or to choose among several approximate matches. Usually the dilution is continued until the unknown is a lighter shade than the standard.

A new Sahli apparatus has been devised to overcome these objections (Fig. 1). The mechanical features have been developed by C. A. Hausser and Son; I have standardized the apparatus. The glass standard is divided into three windows, separated by opaque bands (Figs. 2 and 3) with a 5 per cent variation in the hemoglobin value between each of the three standards. The central window is the standard color, with which the specimen is to be matched (Fig. 4). The lower window is 5 per cent darker, and the upper window is 5 per cent lighter. There are thus always three standards for comparison. When

*From the Cleveland Clinic, Cleveland.

The Sahli-Haden hemoglobinometer is made by C. A. Hausser and Son and is sold by the Arthur H. Thomas Co., West Washington Square, Philadelphia, Pa.

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the correct reading is made, this match is evident from the lighter color above and the darker color below. The colored glass provides a close match for the acid hematin, as shown by spectrophotometric readings.

The color in the graduated tube and that of the glass standard are brought into juxtaposition with a pair of prisms which locate the two color factors in the same focal plane. A square standard tube (Fig. 5) is employed. A magnifier is used to enable focusing past the surface of the calibrated tube and the plane glass surface of the colored glass standard. The inside diameter of the glass tube and the glass standard are checked photoelectrically. An Ulrich pipette (Fig. 6) is used in place of the original Gowers pipette employed with the Sahli apparatus.

Fig. 1.

Fig. 2.

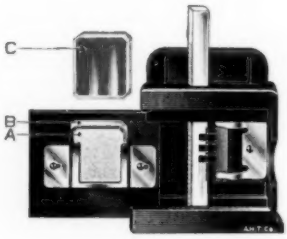

Fig. 3.

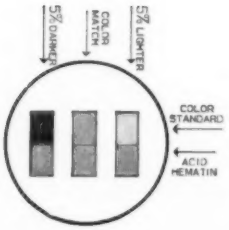

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 1.—Front view with magnifier in position.

Fig. 2.—Front view with magnifier removed, showing color matching rectangles behind glass prisms.

Fig. 3.—Rear view with ground glass or "daylite" plate removed, showing one-half of color matching rectangles.

Fig. 4.—Field of view showing both halves of the three color matching rectangles.

Fig. 5.—Graduated glass tube of square cross section for the acid hematin solution.

Fig. 6.—Ulrich pipette for collecting blood specimens.

Calibration.—The apparatus has been standardized to read in grams of hemoglobin per 100 c.c., by comparing readings with those made on the Haden-Hausser laboratory model hemoglobinometer. No direct comparison has been made with the iron-content and oxygen-content methods, but the Haden-Hausser instrument has been repeatedly proved to give results comparable to those obtained by these two methods.[4]

The comparative readings with twenty different specimens of blood on the two instruments are given in Table I.

PROCEDURE

The Ulrich pipette must be thoroughly dried. Blood is drawn to the 20 c.mm. mark. The tip of the pipette is wiped off, and the bulb is filled with N/10 hydrochloric acid. This is then completely emptied into the graduated tube. If an ordinary 20 mm. pipette is used, N/10 hydrochloric acid is run into the graduated tube in the two graduates, blood is drawn to the graduation mark on the pipette and run into the acid in the tube. The diluted blood should be drawn back into the pipette several times to completely empty it.

TABLE I

SPECIMEN NO.	HEMOGLOBIN IN GRAMS PER 100 C.C. BY HADEN-HAUSSER LABORATORY MODEL HEMOGLOBINOMETER	HEMOGLOBIN IN GRAMS PER 100 C.C. BY NEW SAHLI-HADEN HEMOGLOBINOMETER
	gm.	*gm.*
1	16.0	16.0
2	12.0	11.8
3	9.5	9.4
4	11.0	10.8
5	16.0	16.0
6	14.0	13.8
7	16.0	16.2
8	8.1	8.2
9	16.0	15.8
10	10.5	10.5
11	8.0	7.5
12	9.0	9.0
13	16.0	16.2
14	10.7	10.7
15	8.0	8.0
16	8.0	7.8
17	11.0	10.6
18	8.0	8.2
19	10.5	10.8
20	8.0	8.2
Mean	11.32	11.28

After standing thirty minutes, the color comparison is made. If the reading is made after ten minutes, 4 per cent is added; after fifteen minutes, 3 per cent; and after twenty minutes, 2 per cent. Readings can be made either with daylight, using the ground glass back plate, or with artificial light using a "daylite" glass plate.

In making the color comparison, the magnifier should be focused sharply on the windows of the black mask. Add water with an eye dropper, mixing constantly with the stirring rod until the color of the specimen approximates that of the lowest window. Then add water carefully until the specimen matches the middle window. If the match is correct, the lower window will be darker and the upper window lighter than the specimen.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PREGNANCY. An Investigation of the Patterson Test for the Chemical Diagnosis of Pregnancy, Krieger, V. I. M. J. Australia **1**: 494, 1939.

The author concludes that the Patterson test is the most satisfactory chemical test so far advanced for the detection of early pregnancy.

The Patterson test is carried out in the following way:

Fifty cubic centimeters of concentrated early morning urine, just alkaline to methyl red, are heavily inoculated with *B. coli* and incubated overnight. While the urine is still warm, 0.5 gm. of sodium bisulfite is added, dissolved by being shaken, and allowed to stand for fifteen minutes. The urine is extracted twice with 40 c.c. volumes of ether. The combined ether extracts are washed with water and then shaken with 30 c.c. portions of 10 per cent sodium carbonate until the carbonate layer is quite colorless. The ethereal solution is again washed with distilled water. The ether layer is extracted with two 30 c.c. volumes of N/10 sodium hydroxide. The combined alkali solutions are acidified to Congo red with a 25 per cent solution of sulfuric acid and then extracted with two 40 c.c. volumes of ether. These combined ether extracts are washed with distilled water, with 10 per cent sodium carbonate, and finally with two volumes of distilled water. The final clear, colorless, ether solution is transferred in two portions to a 50 c.c. flask, and the ether is removed by distillation in a water bath at 70° C. The final traces of moisture are removed by application of direct suction to the flask. The dry residue is heated for ten minutes in a boiling water bath with 1 c.c. of phenolsulfonic reagent (9 c.c. of pure carbolic acid and 14 c.c. of concentrated sulfuric acid). The mixture is cooled under the tap, treated with 1 c.c. of a 5 per cent solution of sulfuric acid, and is then reheated in a boiling water bath for two and a half minutes. A positive reaction is indicated by a pink or red color; in the absence of this change, the reading is negative.

ERYTHROCYTE SEDIMENTATION, Practical Value in the Management of Pulmonary Tuberculosis, De Cecio, T., and Elwood, B. J. Am. Rev. Tuberc. **39**: 748, 1939.

The study of the sedimentation rate with a view to establishing its practical value in the management of tuberculosis of the lungs reveals the following facts:

In the initial study of a case, the occurrence of an elevated rate indicates, in a considerable majority of instances, the presence of an active lesion. However, the presence of a normal rate does not exclude an active lesion.

In the correlation of serial rates with definite pathologic trends, the case percentage (55 per cent) of compliance of the rates with the anatomic course is not of sufficient significance to be of practical value.

No greater percentage of relapse or reactivation occurred in the group discharged with elevated rates than in the one with normal rates. Furthermore, a considerable majority of those patients with sustained elevation of the sedimentation rate have remained well from one to five years.

The information obtained from the clinicopathologic study reveals not only all but also more information than can be gleaned from sedimentation rates alone, and with a greater degree of accuracy.

Thus the use of the sedimentation rate in the management of pulmonary tuberculosis as a criterion of activity, course, and prognosis is not of sufficient clinical value to be essential in the care of the tuberculous individual.

BALANTIDIASIS, Young, M. D. J. A. M. A. **113**: 580, 1939.

Seven infections with *Balantidium coli* in white women were observed by the author in South Carolina and 2 cases were found in Tennessee by Meleney.

The infected patients had chronic diarrhea and occasionally dysentery. *Trichuris trichiura, Necator americanus,* and *Strongyloides stercoralis* were present also, and consequently the symptoms may not have been due entirely to the balantidia.

Frequent examinations of the stools were made in cases of infection. Trophozoites were found 127 times in 145 examinations, a percentage of 87.5. Cysts were found 13 times, a percentage of 8.9.

Epidemiologic evidence indicates that the present infections were contracted from human sources rather than from hogs; swine are generally thought to be the source for most infections in man.

The balantidia disappeared from the stools of two patients treated with carbarsone. The stools of one patient were still free after five months. One month had elapsed since the treatment of the second and no balantidia had been seen. Oil of chenopodium given orally was not efficacious.

Few reports of this disease occur in the literature. Previous to this report, 32 cases had been reported from 16 states. This report adds 9 more cases from 2 states.

The evidence indicates that the disease may be more prevalent than is reported.

ENDOCARDITIS, Subacute Bacterial, Evaluation of Sulphanilamide in the Treatment of, Spink, W. W., and Crago, F. H. Arch. Int. Med. **64**: 228, 1939.

Sulfanilamide was administered to 12 patients with subacute bacterial endocarditis. The etiologic agent was *Str. viridans* in 11 patients and *Staph. albus* in one. Of the 2 patients who appeared definitely improved after receiving the drug, one eventually died of the disease, and at the time of writing the other has been well for nine months.

All the patients had bacteriemia. Sulfanilamide rendered the blood stream sterile in 6 of the 12 patients. This bactericidal effect appeared to be only temporary, except in 2 patients.

In 4 patients there was a decline in the temperature coincident with the administration of sulfanilamide. There did not appear to be any relationship between the degree of fever and the therapeutic effect of sulfanilamide.

Although the drug was taken for a long period by several patients, there was a marked decline in the erythrocyte level in only one person. In no patient was a depression of the leucocytes observed.

There was no definite relationship between the amount of free sulfanilamide in the blood and the effect of the drug on the bacteriemia.

The use of sulfanilamide in the treatment of patients with subacute bacterial endocarditis is of doubtful value because of the nature of the focus of infection.

It appears from the foregoing observations that the administration of sulfanilamide to patients with subacute bacterial endocarditis will, in some instances, render the circulating blood free of organisms. Except for 2 patients this bactericidal effect was only temporary and depended on the continued use of the drug. In 2 patients definite improvement followed the use of sulfanilamide. However, the authors believe that sulfanilamide and its related compounds will be of doubtful value in the treatment of patients with subacute bacterial endocarditis because of the very nature of the focus of infection. The proliferating mass of bacteria situated well beneath the surface of the vegetation is probably protected, at least in part, from the action of free sulfanilamide in the blood, as well as of specific antibodies. When the organisms approach the surface of the vegetation, they are carried off in the circulating blood, and under these circumstances may be killed. An analogous therapeutic situation is now recognized in the treatment of patients with bacteriemia due to beta hemolytic streptococci. Lockwood stated that in a number of instances of infection of the blood stream an infected thrombus in a large vessel has prevented satisfactory elimination of the bacteriemia.

Since the presence of sulfanilamide in the blood may have a bacteriostatic effect on some strains of *Str. viridans*, sulfanilamide probably should be administered to any patient with valvular lesions who may be subjected to oral surgical procedures. It is well known that after the extraction of teeth or after a tonsillectomy temporary bacteriemia with *Str. viridans* may result.

CHEMOTHERAPY, Present Position of, by Drugs of the Sulphanilamide Group, Browning, C. H. Brit. M. J., Aug. 5, 1939, p. 266.

While cure of certain experimental general infections with bacteria by sulfanilamide compounds is an enormous advance, the efficacy of the present drugs is by no means ideal. So far at least as mice are concerned, sulfanilamide is surpassed by, for example, M & B 693, rodilone, and especially the related monoacetyl-p-p-diaminodiphenyl-sulfone. But with them all the range of effective dosage is not wide, and treatment to be most successful must be begun early.

Further work on the absorption and excretion of members of the series, and on the chemical transformations which they may undergo in the body, as well as clinical observation, is required in order to enable them to be used to the best advantage as regards the choice of drug, and the dosage in amount and spacing in time. For instance, in treating infection of the meninges by oral or subcutaneous administration it will probably be found that one which rapidly attains a high concentration in the cerebrospinal fluid, such as sulfanilamide, ought to be given in the first place, while more potent but more slowly absorbed compounds, provided they ultimately reach the spinal fluid in sufficient amount, are used to re-enforce and maintain the action.

Where conditions favorable to general infection have arisen, prophylactic use of the sulfanilamide group should be valuable. Where infection has been established, the best therapeutic effects are secured in acute, diffuse conditions, without marked local tissue changes. Hence these drugs are not likely to obviate operative procedures where focal lesions, such as accumulations of pus or extensive necrosis of bone or other tissues, have occurred. Accordingly, powerful antiseptics which act when brought into close contact with the organisms locally, and which are relatively harmless to the tissues, will continue to be required also in the treatment of many septic infections.

SICKLE-CELL ANEMIA, Sedimentation Rates of Sickled and Non-Sickled Cells in, Bunting, H. Am. J. M. Sc. **198**: 191, 1939.

Sickled erythrocytes from patients with sickle-cell anemia and the sickle-cell trait did not form rouleaux and remained almost unsedimented after one hour's time, while nonsickled cells from the same patients formed rouleaux and sedimented.

BIOLOGICAL FLUIDS, Preservation of, Maier, E. J. Bact. **38**: 33, 1939.

A new compound, alkyl-dimethyl-benzyl-ammonium chloride, has been studied in reference to its germicidal, bacteriostatic, and inactive zones in accordance with the term "disinfectant spectrum," as proposed by Marshall and Hrenoff (1937). The effect of this compound in dilutions of 1:4,000 has been studied in the cornea of the rabbit by histologic methods. Comparisons were made with 3.5 per cent tincture of iodine. It was shown that one single application of the iodine tincture was sufficient to destroy the cornea completely and to produce permanent opaqueness in the same. The compound under discussion has been applied to the cornea daily for ten days, with no consequences.

A dilution of 1:50,000 in staphylococcal bacteriophage was found not to interfere with the reproduction of the phage. Furthermore, the phage-disinfectant mixture was found to be capable of reproduction of new phage after three months' contact in the refrigerator. The same concentration of the compound was employed in the preservation of vaccines and venom solutions.

PNEUMONIA, Pneumococcic, Treatment of, with Concentrated Antipneumococcic Rabbit Serum, Wood, W. B., Jr. J. A. M. A. **113**: 745, 1939.

Fifty patients with pneumococcic pneumonia of nine different types were treated with concentrated antipneumococcus rabbit serum. Eight patients with type III pneumonia were treated and 4 died, the serum seeming to be relatively ineffective with this type. For the remaining 42 patients the fatality rate was 14.3 per cent, although 26 per cent of the patients had bacteriemia and a like number had multilobar consolidation. Excluding the patients with type III pneumonia, all the patients who failed to survive had more than one lobe involved, and all had more than five colonies of pneumococci per cubic centimeter in the blood culture except one, an 82-year-old white woman who died of congestive heart failure and uremia. The incidence of empyema was 7 per cent. Thermal reactions were relatively common, occurring in 26 per cent of the patients, and delayed serum sickness developed in more than one-third of the patients.

RABIES in Birmingham, Alabama, Denison, G. A., and Dowling, J. D. J. A. M. A. **113**: 390, 1939.

The data presented are not of a type to warrant definite conclusions. The following general statements are offered as implicit in the experience cited:

Rabies is primarily a veterinary problem. Regardless of its prevalence among dogs, it will probably never cause sufficient morbidity or mortality among human beings in this region to allow its classification as a public health problem of importance.

If rabies were as easily contracted by man as is commonly supposed, it would become one of the leading causes of death in Alabama. Instead, it continues to be a very rare disease.

In this region there appears to be little relationship between mortality from rabies and the administration of antirabies vaccine.

Aside from an actual bite or scratch, circumstances of exposure rarely warrant vaccine treatment.

When considering the advisability of treatment for exposures other than an actual bite, the possibilities of vaccine paralysis or other serious complications should be considered.

The problem of rabies, as it is known in Birmingham, appears to be one of home-owned dogs (pets) and not of unidentified strays.

TUBERCULOSIS: Comparison of Tuberculin Patch Test with Mantoux Intracutaneous Test, Taylor, G. Am. Rev. Tuberc. **40**: 236, 1939.

A total of 25.7 per cent of 744 subjects gave a positive Mantoux and a positive patch test; 68.6 per cent gave a negative Mantoux and a negative patch test. Thus the two tests yielded comparable results in 94.3 per cent of the persons tested.

In 3.6 per cent a negative Mantoux and a positive patch test were observed; of these 27 subjects, 26 were found among young adults, students, and nurses.

In 2.0 per cent a negative patch test and a positive Mantoux were seen; of these 15 subjects, 12, or 80 per cent, were above 50 years of age.

In this series of 744 cases in which the Vollmer patch test was compared with the Mantoux intracutaneous test, using 0.1 mg. O.T., the results indicate that the simpler Vollmer test is equally as effective as the Mantoux test, and possesses many advantages for the physician as well as for the patient.

TUBERCULOSIS: Culture Method in Sputum Examination, Loesch, J., and Petrik, F. Am. Rev. Tuberc. **40**: 233, 1939.

A total of 1,028 sputum examinations on the same number of clinic patients are reviewed.

When the culture method was used to supplement smear and concentration, 24 per cent of the total number of positive cases were detected by culture only.

The economic advantages of this method are self-evident.

It is suggested that all sputa which are negative on direct smear be cultured.

The cultural method used in this study is that described by Petrik (Am. J. Clin. Path. **8**: 134, 1938).

BROMIDE Intoxication, Gundry, L. P. J. A. M. A. **113**: 466, 1939.

Bromide intoxication is a common condition, and, judging from the hundreds of cases which have been reported, it is prevalent in all parts of this country. In spite of its prevalence, many physicians still fail to recognize this condition.

The cause of bromide intoxication is most commonly a physician's prescription written for an alcoholic addict, a psychoneurotic person, or a psychotic patient. If the chloride intake is low, or if the patient is suffering from some debilitating disease, intoxication occurs more readily.

The severity of the symptoms of bromism closely parallels the elevation of the blood bromide level. These symptoms are characteristic and are found in some degree in all patients with a blood bromide content above 150 mg. per 100 c.c.

An alert physician should be able to make the diagnosis from the history and physical examination. The blood bromide should confirm the diagnosis.

The prognosis should be guarded until the underlying condition has been studied.

Treatment of bromide intoxication is divided into preventive and curative measures. The latter is easy and satisfactory, but the former is more important for the physician to remember.

SEDIMENTATION TEST, Choice of Technique for, Hambleton, A., and Christianson, R. A. Am. J. M. Sc. **198**: 177, 1939.

The data obtained at this tuberculosis sanatorium indicate that the sedimentation test remains today essentially what it was when Fahraeus introduced the test in 1918, and that all the significant clinical data which may be obtained by sedimentation procedures can be found by a single one-hour reading by the Westergren technique. Alleged improvements upon the Westergren sedimentation method have been presented which appear theoretically correct, e.g., correction for cell volume, use of heparin in place of citrate, and graphic methods of recording the results. Instead of making the test more valuable, these changes have the reverse effect, and either lead to results of less clinical value, or make the test more tedious to perform or less clear to interpret without increasing its clinical value. Hence it is recommended that the Westergren method, by reason of its simplicity, reliability, and priority, should be adopted as the standard method of performing the sedimentation test.

In conclusion, those who use the Westergren technique should keep in mind that, due to the narrow bore of the tube, it is all the more important to see that the tubes are perfectly vertical during the test.